

BNSDOCID: <WO\_\_B31B161A1\_1\_>

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## RECEPTOR COMPLEXES WITH B29 (IG-BETA OR IG-GAMMA) AND THEIR USES.

The invention described herein relates generally to cellular immunity and the recognition of antigen by cellular immune system components. Such cellular components contain antigen-recognizing receptors which fall generally within the Ig supergene family. The differences between such cellular components, e.g., T- and B-lymphocytes can be utilized in accordance with the teachings contained herein.

BACKGROUND OF THE INVENTION

Humoral immune responses are generally initiated by the binding of antigen to the immunoglobulins present on the membranes of cells. On the cell surface, the antigen and immunoglobulin proteins may react in the presence of one or more non-immunoglobulin proteins, e.g., MB1, and B29, which are implicated in receptor assembly.

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The antigen receptors of B- and T-lymphocytes are members of the Ig supergene family whose specificity is determined by a series of genomic rearrangements. In both cases, the ligand binding domains of the receptors present on these cells are defined by the combination of independently encoded polypeptides that are linked together by disulfide bonds. In addition, both receptors are associated with a number of other proteins on the cell surface.

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It has long been recognized that T-lymphocytes are reactive to compounds which are complexed or presented by Antigen Presenting Cells (APC) and/or Major Histocompatibility Complex (MHC). Normally T-lymphocytes recognize antigen which is presented on the surface of APCs in the context of MHC. As such, MHC may trigger antigen recognition by the T-lymphocytes.

MHC is comprised of numerous molecules which tag and "activate" the antigen, causing recognition by the T-cell receptors, binding and ultimately internalization of the antigen by the cell.

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The T cell receptor (TCR) is comprised of at least seven different polypeptides that include the antigen specific alpha and beta chains, the zeta chain, which is a signaling molecule, and the gamma, delta and epsilon chains, which form a second signaling component. All are required for efficient assembly and surface transport (Weiss and Stobo, 1984; Ohashi et al., 1985; Berkhout et al., 1988; Sussman et al., 1988). Comparable proteins to the T-cell receptor associated proteins in B-cells include MB1 and B29, also known as IgMa and IgMb respectively (Hermanson et al., 1988; Hombach et al., 1988; Sakaguchi et al., 1988; Hombach et al., 1990; Campbell et al., 1991). MB1 and B29 form a disulfide linked heterodimer that is associated with IgM (Hombach et al., 1990), and both proteins share a negatively charged intracytoplasmic sequence motif with CD3  $\eta$ ,  $\delta$  and  $\epsilon$  (Reth, 1989). MB1 and B29 appear to be required for transport of IgM to the plasma membrane of transfected fibroblasts and B-cells (Hombach et al. 1990; Venkitaraman et al. 1991).

Experiments with mutant T-cell lines and isolated TCR components have established that the  $\epsilon$  chain is both necessary and sufficient for signal transduction (Weissman et al., 1989; Frank et al., 1990; Irving and Weiss, 1991; Romeo and Seed, 1991). In addition the CD3  $\epsilon$  chain is also capable of inducing T cell activation possibly by an alternative pathway (Letourneur and Klausner, 1992).

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Crosslinking of the T-cell receptor, or the isolated  $\epsilon$  or  $\delta$  chain, leads to CD45-dependent activation of protein

kinases, increased phosphoinositol turnover and calcium mobilization. A very similar set of events is induced by the crosslinking of IgM on the surface of B-cells; however, the functional role of the IgM associated proteins has to date been inadequately defined.

It has long been desired to modify T-lymphocyte receptor reactivity to eliminate the need for MHC and/or APCs.

Consequently, one object of the present invention is therefore to define the role of the IgM-associated non-immunoglobulin proteins.

Another object of the present invention is to provide a method of modifying T-lymphocyte immunity to render such immunity essentially MHC non-dependent.

Yet another object of the present invention is to provide methods of assessing T- and B-lymphocyte and immune system activity, including, e.g., antigen recognition, MHC complexation or reactivity, B-cell surface IgM activity and other factors.

These and other objects will be apparent to those of ordinary skill from teachings herein.

#### SUMMARY OF THE INVENTION

A method of modifying cellular immunity is disclosed comprising transfecting a T-lymphocyte with B-cell derived immunoglobulin and B29.

The transfected T-lymphocytes are included herein, as are various methods of use.

Also included are mast cells and macrophage cells transfected with the B-cell derived immunoglobulin and B29.

- 5 Also included in the invention are the DNA and RNA constructs described herein, as well as antibody constructs which recognize such molecules, the immunoglobulins present on the surface of the T-lymphocytes and non-Ig proteins.

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BRIEF DESCRIPTION OF THE DRAWINGS

The invention is described in detail in conjunction with the following drawings.

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FIGURE 1: DNA constructs, and cell surface expression of human immunoglobulin

- (A) Maps of immunoglobulin, B29 and MB1 expression  
20 vectors. The Spleen Focus Forming Virus-Long Term Repeat (SFFV LTR) promoter was used in all constructs for T cell expression. Human growth hormone (hGH) introns and polyadenylation signals were added to both B29 and MB1 cDNAs, and a histidinol resistance gene was included in  
25 the MB1 plasmid.

A phosphorylcholine (PC) specific mouse variable region from S107, was combined with a human heavy chain constant region engineered to be synthesized only in the membrane  
30 bound form. The light chain was composed of an S107 kappa variable region and the human kappa constant region. The heavy chain and light chain were on the same plasmid as a neomycin resistance gene.

- 35 (B) Flow cytometric analysis of surface expression of human IgM on transfected Jurkat cells. The relative cell number is plotted against fluorescence intensity on a

logarithmic scale. The constructs transfected, cell line and the number of times the cell line was enriched by sorting (SX\_) are indicated at the top of each panel. "Control" indicates unstained cells, and GAHIGM indicates staining with FITC conjugated goat anti-human IgM. SFFV: Spleen focus forming virus long terminal repeat; B29: B29 cDNA; MB1: MB1 cDNA; hGH: human growth hormone splice and polyadenylation signals; His: histidinol resistance gene; mIgM: phosphorylcholine specific, membrane bound form of human IgM heavy chain; kappa: phosphorylcholine specific kappa light chain; Neo: neomycin resistance gene; #: cell line number; SX\_: the number of times a cell line was enriched for surface IgM by sorting; p467: B29 expression vector; p474: MB1 expression vector; p468: IgM expression vector.

**FIGURE 2: Expression of IgM protein, B29 and MB1 mRNAs**

(A) Silver stained SDS acrylamide gel of affinity purified phosphorylcholine binding immunoglobulin from transfected Jurkat cells and controls.

(B) Northern analysis of B29 and MB1 expression in the transfected cell lines. The cell types, constructs transfected, and cell line number (FIGURE 1B) are indicated at the top of each lane. IgM was the starting cell line for production of IgM#3; it had 1-2% surface IgM expression; m and k indicate the position of the heavy and light chains determined from purified standards; Kb: kilobase pairs; Kd: kilodaltons.

**FIGURE 3: Affinity purification of surface iodinated proteins from transfected cell lines**

Phosphorylcholine binding immunoglobulins and associated polypeptides were affinity purified from 1% digitonin lysates of surface iodinated cells. The cell types,

constructs transfected and cell line number are indicated at the top of each lane, the same as in FIGURE 2A; m and k indicate the position of the heavy and light chains determined from purified standards; Kd: kilodaltons.

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**FIGURE 4:** Calcium flux assays of transfected Jurkat cells

Cells loaded with Fura-2 were assayed fluorimetrically for calcium mobilization in response to 1.5 ug/ml anti-CD3, 10 ug/ml anti-human IgM, 10 ug/ml isotype control monoclonal antibody or 500 ng/ml of phosphorylcholine coupled to bovine serum albumin (PC-BSA). The cell types and constructs transfected are indicated at the left and the reagents added are indicated at the top.

Symbols: IgG1: isotype control monoclonal antibody; a-CD3: anti-CD3 antibody; a-IgM: anti-IgM antibody; PC-BSA: phosphorylcholine coupled to bovine serum albumin; Jurkat: untransfected Jurkat cells; IgM: Jurkat cells transfected with IgM #3 (FIGURE 1B); IgM+B29: Jurkat cells transfected with IgM+B29 #4 (FIGURE 1B); IgM+B29+MB1: Jurkat cells transfected with IgM+B29+MB1 #9 (FIGURE 1B); time and  $[Ca^{+2}]$  are indicated at the bottom right.

**FIGURE 5:** Generation of inositol triphosphates (IP3) by transfected Jurkat cells

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Cells metabolically labeled with  $[^3H]$ myoinositol were stimulated with anti-CD3, anti-human IgM or an IgG1 isotype control antibody, and inositol triphosphate (IP3) was measured by extraction and anion exchange chromatography. The percent of unstimulated IP3 production is plotted on the Y axis. Error bars indicate the standard deviation from the mean (n=2). Symbols:

control: no primary antibody; other symbols as in FIGURE 4.

FIGURE 6: IL-2 secretion in response to anti-IgM by transfected Jurkat cells

Cell lines were stimulated with anti-CD3, anti-human IgM or an IgG1 isotype control antibody in the presence of phorbol-myristate acetate (PMA) or PMA alone. The mean +/- the standard deviation (n=3) of the number of U/ml of IL-2 secreted for two independent experiments is indicated on the Y axis. (Biological Response Modifiers Program (ICN Biomedical Inc.)) Symbols: PMA: phorbol myristate acetate; IgM+B29: Jurkat cells transfected with IgM+B29 #5 (FIGURE 1B); other symbols as in FIGURE 4.

FIGURE 7: IL-2 secretion in response to phosphorylcholine by transfected Jurkat cells

Cell lines were unstimulated or stimulated with bovine serum albumin (BSA) or phosphorylcholine coupled to bovine serum albumin (PC-BSA). The mean +/- the standard deviation (n=3) of the number of U/ml of IL-2 secreted for two independent experiments is indicated on the Y axis (Biological Response Modifiers Program (ICN Biomedical Inc.)). Symbols: BSA: bovine serum albumin; IgM+B29: Jurkat cells transfected with IgM+B29 #5 (FIGURE 1B); other symbols as in FIGURE 4.

FIGURE 8: Dose response to PC-BSA and blocking by monomeric phosphorylcholine in transfected Jurkat cells

(A) Cells loaded with Fura-2 were assayed fluorimetrically for calcium mobilization in response to increasing doses of PC-BSA. The calcium concentration (Y axis) was calculated as indicated in the experimental procedures and plotted against concentration of

PC-albumin (X axis) on a logarithmic scale. The constructs transfected and cell line #'s are indicated. Diamonds represent IgM+B29+MB1 transfected Jurkat cells, and squares indicate IgM+B29 transfected Jurkat cells.

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(B) IgM+B29 transfected cell line #5 was stimulated with 2.5mM phosphorylcholine followed by 500ng/ml PC-BSA and finally with 5mg/ml of anti-CD3 monoclonal antibody as indicated by the arrows. Symbols: PC: monomeric  
10 phosphorylcholine; other symbols as in FIGURE 4.

FIGURE 9: Assays of alternative cell lines transfected with IgM, B29 and MB1.

(A) Macrophage cell line P388D1 transfected with  
15 IgM, B29 and MB1. Stimulation was accomplished with phosphorylcholine-BSA.

(B) Mo cell line P388D1 transfected with IgM, B29 and MB1. Stimulation was accomplished with anti-human IgM.

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FIGURE 10: Assays of Mast cells transfected with Igm, B29 and MB1.

(A) Stimulation of Mast cells P815 stimulated with anti-IgM.

25 (B) Stimulation of Mast cells P815 transfected with IgM, B29 and MB1, and stimulated with phosphorylcholine-BSA.

(C) Mast cells P815 control.

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#### DETAILED DESCRIPTION

This invention relates to the receptors and related molecules on the surface of humoral immune system cellular components, e.g., T- and B-lymphocytes, DNA  
35 molecules and derivatives thereof, e.g., mRNA, which code on expression for these receptor compounds, sense and anti-sense RNA molecules which likewise code for such

molecules or for other molecules, antibodies which recognize such proteins and nucleic acid derivatives, antibodies that recognize B-cell receptors and receptor related molecules individually and in combinations such as complexes, as well as anti-idiotypic antibodies which recognize both these antibodies and ligands, including agonists, antagonists and the like to these B-cell receptor and receptor related molecules. Numerous methods of production and use are also included.

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A preferred aspect of the invention is drawn to a method of modifying T-lymphocytes to render said cells substantially non-dependent upon the presence or activity of MHC.

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Another preferred aspect of the invention is drawn to a method of modifying mast cells or macrophage cells to render said cells active in recognizing antigen in the presence or absence of MHC.

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Another preferred embodiment of the invention relates to the DNA constructs described herein and in the plasmids incorporating such constructs.

25 Another preferred embodiment of the invention relates to a unicellular host transfected with the plasmids described herein.

Another preferred embodiment of the invention relates to the antibodies which are described herein, including polyclonal, monoclonal and chimeric antibodies. One preferred chimeric antibody contains an immunoglobulin protein or fragment in combination with a B29 protein or fragment of B29. Another preferred chimeric antibody contains an immunoglobulin molecule or fragment and a protein molecule or fragment other than B29, such as those described in NATURE, Vol. 338:383 (March 30, 1989).

Many of these chimeric antibodies will contain the consensus amino acid sequence noted therein.

In accordance with this detailed description, the following definitions apply:

Expression control sequence: a DNA sequence that controls and regulates the transcription and/or translation of another DNA sequence.

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Operatively linked: a DNA sequence is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) upstream (in front of) the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

25

Antibody: an immunoglobulin molecule or functional fragment thereof, such as FAb, F(ab')<sub>2</sub>, or dAb. An antibody preparation is reactive for a particular antigen when at least a portion of the individual immunoglobulin molecules in the preparation recognize (i.e., bind to) the antigen. An antibody preparation is non-reactive for an antigen when binding of the individual immunoglobulin molecules in the preparation to the antigen is not detectable by commonly used methods. The fragments of the antibodies described herein, in particular, include the phosphorylcholine specific mouse variable region from S107, the human heavy chain constant region, a derivative

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of the human heavy chain constant region in membrane bound form, and the light chain S107 kappa variable region, alone and operatively linked to a human kappa constant region. Hence, chimeric bodies are likewise  
5 included.

Standard hybridization conditions: salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash.

10

DNA sequence: polynucleotide sequences prepared or isolated using recombinant DNA techniques. These include cDNA sequences, DNA sequences isolated from their native genome and synthetic DNA sequences. The term as used in  
15 the claims is not intended to include naturally occurring DNA sequences as they exist in nature.

Receptor and receptor complex: includes both the singular and plural, and contemplates the existence of  
20 one or more structures comprised of the protein(s) which make up the ligand recognition site. As such, one or more proteins may be involved, as well as one or more compounds not directly involved in ligand recognition, e.g., MB1, B29 and other compounds, which even though not  
25 included in the recognition reaction, are required, preferred or are typically present when the recognition reaction occurs. All such compounds are included, taken individually as well as in combinations.

30 Expression of recombinant molecules as used herein may involve the post-translational modification of a resultant polypeptide coded by the sequence present in the host cell. For example, in mammalian cells, expression might include, among other things, the  
35 production of an mRNA molecule or a polypeptide, glycosylation, lipidation or phosphorylation of the polypeptide, or cleavage of a signal sequence to produce

a "mature" protein. Accordingly, as used herein, the term "polypeptide" encompasses full-length polypeptides, fragments of mature proteins and modifications or derivatives thereof, such as glycosylated versions of such polypeptides, polypeptides retaining a signal peptide, truncated polypeptides having comparable biological activity and the like.

Also as noted above, expression of a "derivative" of the sequence may involve the production of an intermediate molecule, which is generated in during the expression of the protein. Typically, this involves the expression of mRNA which likewise codes for the particular polypeptide to be ultimately expressed. In this instance, the mRNA molecule is deemed to be a derivative of the DNA coding sequence contained in the particular expression vector.

Signal and signal transduction as used herein, refer to changes which occur in the cell in response to crosslinking (binding) of the receptor by reaction with a ligand. Examples of such cellular changes include initiation of a cascade of enzyme reactions, rapid increases in calcium flux, increase in phosphoinositol (IP3) turnover and secretion of lymphokines from lymphocytes.

As an initial step in demonstrating the structural and functional requirements for signal transduction by immunoglobulin, the immunoglobulin antigen receptor in T-lymphocytes (Jurkat T cell line) was reconstituted by transfection of cloned components derived from B-lymphocytes. The transport of IgM to the surface of T-cells required the co-expression of the immunoglobulin heavy and light chains with B29. In addition, the transfected receptor was fully active in the presence of B29. MB1, a second IgM associated polypeptide, was not required for either transport or signal transduction.

Immunoglobulin receptor function was "reconstituted" by transfection of the B-cell cloned receptor components into human T-lymphocytes, and it was noted that the transport of IgM to the surface of the T-cells ("host") required the co-expression of B29. Furthermore, the expression of IgM and B29 in combination was sufficient to reconstitute antigen specific signal transduction by the expressed immunoglobulin in the transfected T-cells. This established a functional requirement for B29, and suggests that the signaling apparatus of T- and B-cells is structurally homologous.

#### Surface expression of IgM in T-cells

Phosphorylcholine specific immunoglobulin receptor components were expressed in T-cells by stable transfection of DNA constructs based on a spleen focus forming virus-long terminal repeat (SFFV-LTR) promoter (Saito et al., 1987) (FIGURE 1A). A human IgM heavy chain minigene that directs the synthesis of membrane anchored IgM (Danner and Leder, 1985) was combined with a phosphorylcholine specific heavy chain variable region (Crews et al., 1981). The light chain was composed of the corresponding kappa variable region (Kwan et al., 1981), coupled with a human kappa constant region gene (Hieter et al., 1982). To insure coordinate expression of the immunoglobulin heavy and light chains the two transcription units were combined in a single plasmid with a neomycin resistance gene. Human growth hormone (hGH) polyadenylation and splice consensus sequences (Saito et al., 1987) were added to mouse MB1 and B29 cDNAs, and a histidinol resistance gene was included as a second drug resistance marker in the MB1 expression vector (Hartman and Mulligan, 1988).

IgM expression was readily detected on the surface of Jurkat cells transfected with IgM and B29, or a combination of IgM, MB1 and B29. In contrast, it was

difficult to detect IgM on Jurkat cells transfected with the heavy and light chain alone, or a combination of IgM and MB1. In all cases we enriched for surface IgM positive cells by selection with a fluorescence activated cell sorter (FIGURE 1B). High levels of surface IgM expression were achieved after 1-2 rounds of selection of cell lines transfected with either IgM and B29, or IgM, B29 and MB1. However, eight rounds of sorting were required for cell lines transfected with IgM alone, and the combination IgM and MB1 was always negative (FIGURE 1B).

The products of the transfected phosphorylcholine specific immunoglobulin genes were appropriately assembled by affinity purification with phosphorylcholine (PC) coupled to sepharose (PC-sepharose). Equivalent amounts of both heavy and light chains were obtained from all cell lines and clonal derivatives regardless of the level of surface expression (FIGURE 2A). Thus the level of surface IgM expression was not simply a function of the amount of IgM synthesis. In addition, the steady state levels of transfected B29 and MB1 mRNA were comparable to a B-cell control (FIGURE 2B). The mRNAs produced from the transfected genes were somewhat larger than their B-cell counterparts since they contained additional human growth hormone sequences. We concluded that the combination of heavy chain, light chain and B29 was sufficient for efficient transport of IgM to the surface of T-cells. In contrast, powerful selection was required to obtain surface IgM positive cells in the absence of B29.

#### Transfected IgM is associated with B29 and MB1

To determine whether B29 and MB1 were associated with IgM, we iodinated transfected T-cell lines and affinity purified phosphorylcholine binding IgM and associated molecules with PC-sepharose from digitonin lysates

(FIGURE 3). A polypeptide with the appropriate electrophoretic mobility for B29 (44 Kd) was co-purified with immunoglobulin heavy and light chains from Jurkat cells transfected with IgM and B29. The same polypeptide and three additional species were co-purified from the IgM, B29 and MB1 transfectants (Fig 3). The 32Kd band was consistent with MB1. The additional bands at 52Kd and 39Kd may be alternate forms of B29 and MB1, or T-cell encoded proteins.

10

As a control the same experiments were performed with Jurkat cells that expressed surface IgM alone (cell line #3, Fig 1B). Although we were able to purify iodinated immunoglobulin heavy and light chains from the IgM control, there were no associated proteins (FIGURE 3). B29 was therefore associated with IgM on the surface of T-cells, and this interaction occurred in the absence of MB1. Furthermore, when both MB1 and B29 were present in the transfected T-cells, both were associated with IgM.

20

Crosslinking of IgM with anti-receptor antibodies induces calcium mobilization, phosphoinositol turnover, and Il-2 secretion

The function of the transfected immunoglobulin was initially assessed by measurement of calcium flux in response to receptor crosslinking. Fura-2 loaded cell lines were treated with a monoclonal anti-human IgM antibody, a monoclonal anti-CD3, or an isotype matched monoclonal antibody control. Jurkat cells transfected with either IgM and B29, or IgM, B29 and MB1 responded to anti-IgM crosslinking with a rapid increase in free intracellular calcium (FIGURE 4). Untransfected Jurkat cells, and Jurkat cells that expressed high levels of IgM alone did not respond to anti-IgM crosslinking, but were fully competent to respond to anti-CD3 (FIGURE 4). In addition, the isotype control IgG1 antibody had no effect

(FIGURE 4), and polyclonal goat anti-IgM antibodies had the same effect as monoclonal anti-IgM (not shown).

Inositol phosphate turnover, triggered by the activation of phospholipase C, is another measure of signal transduction by immunoglobulin. To confirm and extend the results obtained in the calcium flux assays, inositol turnover was measured in response to anti-IgM antibody (FIGURE 5). Crosslinking immunoglobulin on the surface of T-cell lines transfected with IgM and B29, or IgM, B29 and MB1, resulted in an increase in cellular inositol-3-phosphate (IP3). In the same experiments, anti-IgM antibody had no effect on untransfected Jurkat cells (FIGURE 5). Thus, the combination of immunoglobulin and B29 expressed on the surface of T-cells was fully functional, and activated intracellular calcium mobilization and phosphoinositol turnover.

One biological effector function induced by engagement of the T-cell receptor is secretion of IL-2. To determine whether transfected immunoglobulin activates this downstream response in T-cells, IL-2 production was measured in response to anti-IgM (FIGURE 6). Jurkat cells that expressed either IgM and B29, or IgM, B29 and MB1 secreted IL-2 in response to anti-IgM treatment. This response was specific; untransfected Jurkat cells did not respond to anti-IgM. Furthermore, the same control cell line was competent to produce IL-2 in response to anti-CD3 stimulation. None of the lines were induced with the isotype control monoclonal antibody (FIGURE 6).

#### Signal transduction induced by antigen

As described above, T-lymphocytes respond to processed peptides associated with MHC on the surface of other cells, whereas B-cells respond to native antigens. To determine whether Jurkat cells transfected with

anti-phosphorylcholine specific IgM would recognize antigens in the absence of MHC, transfected Jurkat cells were challenged with phosphorylcholine coupled to bovine serum albumin (PC-albumin) (Figs. 4, and 7). The  
5 challenge with PC-albumin induced calcium flux and IL-2 secretion in the T-cell lines that expressed B29 in addition to IgM, whereas controls with albumin alone were negative. Once again, in antigen stimulation experiments, T-cells that expressed surface IgM alone  
10 failed to respond (FIGURE 4).

The potential additive or synergistic effects of MB1 were assessed in dose response experiments (FIGURE 8A). Cell lines expressing equivalent amounts of surface IgM had  
15 similar responses to increasing concentrations of PC-BSA whether or not MB1 was present. In both cases, a response was obtained with as little as 0.5ng/ml of PC-albumin, and a plateau was reached at 0.1-0.5mg/ml. The response decreased at doses greater than 1mg/ml,  
20 which was consistent with the possibility that signaling was dependent on receptor crosslinking.

The role of receptor crosslinking in signaling by stimulating cells with monomeric phosphorylcholine was  
25 therefore further examined (FIGURE 8B). The monomeric antigen did not induce signaling, but was able to block responses induced with PC-albumin (FIGURE 8B). Thus, crosslinking of the transfected immunoglobulin appears to be an important feature of the signaling mechanism.  
30 The finding that immunoglobulin is associated with several other proteins on the plasma membrane of B-cells raises a number of interesting questions about the role of these accessory molecules in receptor assembly and signal transduction. Co-expression of IgM and B29 is  
35 sufficient to reconstitute both immunoglobulin surface expression and function in T-cells.

There is persuasive evidence that two of the immunoglobulin associated proteins, MB1 and B29, are important for the transport of immunoglobulin to the surface of B-cells and fibroblasts. B-cell lines that  
5 lack MB1 did not express surface IgM, and this phenotype can be restored by transfection of cloned MB1 (Hombach et al., 1990). Thus, MB1 is required for receptor assembly in B-cells. Similarly, in fibroblasts, both MB1 and B29 are required for surface expression of IgM. However,  
10 other immunoglobulin isotypes can be expressed on the surface of fibroblasts with B29 even in the absence of MB1 (Venkitaraman et al., 1991). One model IgM receptor structure proposes that IgM interacts with a pair of MB1 and B29 heterodimers. In this model, MB1 and B29 are  
15 disulfide linked and interact with IgM in part via polar amino acids in the transmembrane domain. In view of this proposed structure, it was surprising to find that MB1 was not required for either surface expression or function of IgM antigen receptors on transfected T-cells.  
20 Failure to obtain functional reconstitution of the antigen receptor with MB1 alone was not simply due to the lack of transfected gene expression (FIGURE 2B), or to an inactive MB1 protein, since MB1 was associated with IgM and B29 in T-cells transfected with all of these  
25 components (FIGURE 3). Furthermore, addition of MB1 to cell lines that expressed B29 and IgM, but had low levels of surface IgM, induced high levels of surface immunoglobulin (not shown). One interpretation is that one or more T-cell components which are not present in  
30 fibroblasts or B-cells can substitute for MB1.

Progress in understanding the functional role of the immunoglobulin receptor subunits in signaling has been hindered by the multi-subunit nature of the receptor. In  
35 addition, transfected immunoglobulin expressed on the surface of fibroblasts does not appear to be functional for signal transduction even in the presence of B29 and

MB1 (Venkitaraman et al., 1991). The ability to produce a functional receptor by transfection in T-cells establishes that there is structural similarity between the T- and B-cell signal transduction pathways. This observation should greatly simplify the structural and functional analysis of the IgM antigen receptor.

The B-cell derived IgM receptor can interact with the T-cell signal transduction apparatus. There are many similarities between the signaling pathways of these two receptors as well as several significant differences. For example, much experimental evidence points to activation of tyrosine kinases as one of the first steps in the signal transduction pathways of the TCR (reviewed by (Klausner and Samelson, 1991)), and (Campbell and Sefton, 1990; Gold et al., 1990). Both B29 and MB1 are rapidly phosphorylated upon IgM crosslinking in B-cells (Campbell and Sefton, 1990; Campbell et al., 1991; Gold et al., 1991), and both  $\zeta$  and ZAP-70 are phosphorylated upon TCR crosslinking in T-cells (Baniyash et al., 1988; Chan et al., 1991). However the specific kinases that are co-immunoprecipitated with IgM in B-cells (*p56lyn*, *fyn* and *blk*) (Burkhardt et al., 1991; Yamanashi et al., 1991), differ from those that are associated with the TCR (*fyn*) (Samelson et al., 1990).

There are at least two mechanisms by which IgM and B29 could overcome the differences between the signal transduction apparatus of T- and B-cells. First, these molecules could interact directly with and activate one of the many T-cell protein tyrosine kinases by forming a complex with structural similarity to CD3  $\zeta$  or  $\epsilon$ . Crosslinking of the isolated  $\zeta$  or  $\epsilon$  chains is sufficient to induce signaling in transfected T-cells (Irving and Weiss, 1991; Romeo and Seed, 1991; Letourneur and Klausner, 1992), and there is sequence homology between  $\zeta$ ,  $\epsilon$  and B29 (Reth, 1989). Mutations in the shared

consensus sequence destroy signal transduction by the e chain.

A second mechanism which explains signal transduction by  
5 IgM and B29 in T-cells involves the direct association of  
IgM with T-cell encoded proteins that in turn make the  
appropriate cellular connections. The CD3 components are  
particularly appealing candidates for this function since  
they are both structurally and functionally related to  
10 B29 and MB1 (Reth, 1989).

Additional polypeptides were detected in the IgM, B29 and  
MB1 transfectants, but no significant consequences for  
calcium flux, inositol metabolism or IL-2 secretion were  
15 observed. (FIGURES 4, 5, 6, 7 and 8). In addition, our  
experiments suggest that B-cell lines may be used to  
functionally reconstitute the T-cell receptor from cloned  
components.

20 Another major difference between the T-cell receptor and  
immunoglobulin antigen receptors is the nature of the  
antigen recognized by the two receptors. Immunoglobulins  
recognize antigens directly, whereas recognition of  
antigen by the TCR requires the presence of MHC. The  
25 requirement for MHC in conjunction with or prior to T-  
cell recognition places severe limitations on the targets  
recognized by T-cells, and makes transfer of cellular  
immunity MHC restricted. For this reason, there has been  
considerable interest in modifying T-cell recognition to  
30 abrogate the MHC requirement.

Several groups have shown that chimeric receptors  
composed of immunoglobulin variable regions and TCR  
constant regions can function in an MHC independent  
35 fashion. However this approach is limited by the  
formation of Vh-Cb homodimers, and pairing of the heavy  
and light chain variable regions has not been successful

to date. (Kuwana et al., 1987; Becker et al., 1989; Gross et al., 1989; Goverman et al., 1990).

Another approach has been used to produce z-CD4 chimeric proteins that were capable of directing T-cells to targets that express HIV envelope proteins (Romeo and Seed, 1991). In this system, antigen binding results in T-cell activation via z-CD4 crosslinking.

Our reconstitution experiments offer a different solution to the difficult problem of MHC restriction in the transfer of cellular immunity. T-cells that express functional immunoglobulin antigen receptors on the cell surface have the potential for recognizing any antigen recognized by antibodies in an MHC independent fashion.

To determine whether the procedures described above were dependent on the function of T lymphocytes, immunoglobulin IgM and the B cell derived receptor molecules, MB1 and B29, were transfected into cloned mast cells and macrophages. These constructs were then stimulated with antigen, phosphorylcholine-BSA (PC-BSA) or anti-human IgM antibodies. The results are shown in Figures 9 and 10. The ability to produce a functional receptor by transfection in these other cells indicates that there is structural and functional similarity in signal transduction between these various cell types. Hence, these other cell lines can be used to functionally reconstitute the T-cell receptor from cloned components.

DNA sequences and constructs can be made as described in the Example which code for the B-cell derived immunoglobulin and related proteins. Such constructs can be inserted into host organisms to induce expression of these proteins.

From the DNA sequences, one can readily ascertain the amino acid sequences of these B-cell derived proteins. IgM, B29, MB1 and other related proteins, such as the isolated antibody polypeptide chains. This has been  
5 accomplished, and it also may be possible to make substantial alterations in the polypeptide sequence, including insertions as well as deletions, to obtain a variety of molecules which have substantially the same biological activity or immunological activity profile.

10

For example, one may be able to use ligand DNA sequences to produce nucleic acid molecules which intervene in ligand expression at the translational level. This approach may utilize antisense nucleic acids and  
15 ribozymes to block translation of specific mRNA, either by masking mRNA with an antisense molecule or cleaving it with a ribozyme. In this manner, coding and expression of these proteins can be inhibited.

20 Antisense DNA and RNA molecules are complementary to at least a portion of specific mRNA molecules. They may hybridize to form double stranded mRNA. The cell does not translate mRNA in this double stranded form, thus interfering with expression of the mRNA coded protein.  
25 Such anti-sense methods have been used to inhibit gene expression *in vitro*.

Ribozymes are RNA molecules which have the ability to cleave other single stranded RNA molecules, roughly  
30 analogous to DNA restriction endonuclease. Ribozymes were discovered in RNA molecules which excise their own introns. By modifying these RNA molecules, it is possible to produce specific nucleotide sequences which are recognized and cleaved. Because they are sequence  
35 specific, only particular mRNAs such as those which code for the B-cell derived proteins would be inactivated.

Another feature of the invention involves the expression of the DNA sequences for B-cell derived IgM, MB1 and B29. DNA sequences may be expressed by operatively linking them to an expression control sequence, inserting into an  
5 appropriate expression vector and employing that expression vector to transform an appropriate host.

Such operative linking of a DNA sequence of this invention to an expression control sequence, of course,  
10 includes, if not already part of the DNA sequence, the provision of an initiation codon, e.g., ATG, in the correct reading frame upstream of the coding DNA sequence.

15 A wide variety of expression vector/host combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include  
20 derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col E1, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage  $\lambda$ , e.g., NM989 and other phage DNA, e.g., M13 and Filamentous single stranded  
25 phage DNA; yeast plasmids such as the 2 $\mu$  plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells, vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ  
30 phage DNA or other expression control sequences, and the like.

In selecting the appropriate expression control sequences, a variety of factors will normally be  
35 considered. These include, for example, the relative strength of the system, its controllability, its compatibility with the particular DNA sequence or gene to

be expressed, particularly with regard to potential secondary structures.

Many unicellular host cells are useful in expressing the  
5 DNA sequences used in the invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green  
10 Monkey kidney cells (e.g., COS 1, BSC1, BSC30 and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

Suitable unicellular hosts will be selected by  
15 consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, the toxicity to the host of the product encoded by the DNA sequence(s) to be  
20 expressed, and the ease of purification of the expression products.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to  
25 express the DNA sequences used. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the  
30 desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other  
35 proteins encoded by the vector, such as antibiotic markers, will also be considered.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the appropriate DNA sequences on  
5 fermentation or in large scale animal culture.

It is possible to isolate the mRNA from cells which are expressing the appropriate IgM or related proteins and to create a cDNA library from them. Many methods are known  
10 for isolating mRNA and producing cDNA from it.

The cDNA can be inserted into the appropriate vector, such as the eukaryotic expression vector pCDM8. This plasmid has certain advantages, including a high copy  
15 number in E.coli, a eukaryotic promoter and a high level of expression. However, other vector expression systems can be used.

After constructing a cDNA library, clones can be isolated  
20 which contain cDNA sequences coding for B-cell derived IgM, B29, MB1 and other molecules. These are several ways to isolate cDNA for a differentially expressed mRNA, for example +/- screening with labeled cDNA, production of subtracted cDNA libraries, screening with subtracted  
25 cDNA probes, etc.

A variety of expression techniques can likewise be used. For example, antibody screening of proteins encoded by cDNA cloned into the vector, activity assays of  
30 conditioned media after injection of RNA from cloned cDNA or plasmid/phage carrying promoter or other indicator sequences may be evaluated.

Transfection can also be accomplished by a variety of  
35 methods. For example, spheroblast fusion, DEAE dextran and electroporation can be used, primarily for transient

expression. Stable expression typically utilizes calcium phosphate, spheroblast fusion and electroporation.

Partial DNA sequences corresponding to the partial amino  
5 acid sequences of the proteins of the present invention  
or a portion thereof, or a degenerate variant of such  
partial DNA sequences, may be prepared as probes to  
screen for complementary sequences and genomic clones in  
the same or alternate species, such as humans. The  
10 present invention thus extends to the probes so prepared  
that may be provided for screening cDNA and genomic  
libraries for clones that may correspond to genes  
expressing the respective proteins. For example, the  
probes may be prepared with a variety of known vectors.  
15 The present invention also includes the preparation of  
the plasmids including such vectors.

The receptor proteins are ideally prepared by the  
recombinant techniques described herein, isolation and  
20 purification from cells known to bear or produce the  
receptor proteins, such as B-lymphocytes. The cells or  
active fragments likely to participate in receptor  
protein synthesis or to have receptor protein associated  
therewith may be subjected to a series of isolation  
25 techniques, such as for example elution of detergent-  
solubilized proteins from an affinity matrix, whereupon  
the present receptor proteins may be recovered.  
Naturally, alternate procedures for preparation of the  
receptor component proteins are contemplated, and the  
30 invention is not limited to the procedures set forth  
herein.

The present invention also extends to antibodies  
including polyclonal, monoclonal and chimeric, which are  
35 comprised of or recognize the IgM receptor and the non-  
immunoglobulin proteins described herein. These  
antibodies may find use in a variety of research,

diagnostic and therapeutic applications. For example, the antibodies can be used to screen expression libraries to obtain the gene that encodes either the receptor complex or non-Ig proteins. Further, those antibodies  
5 that neutralize receptor component activity can be employed in intact animals to better elucidate the biological role that the receptor and the related proteins play. Such antibodies can also participate in drug screening assays to identify drugs or other agents  
10 that may exhibit the same or a contrary activity as the proteins.

Polyclonal, monoclonal and chimeric antibodies to the receptor and components thereof are thus contemplated.  
15 These molecules are capable of preparation by known techniques such as the hybridoma technique, utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Immortal, antibody-producing cell lines can be created by techniques other than fusion, such as direct  
20 transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Naturally, these antibodies are merely illustrative of antibody preparations that may be made in accordance with the present invention.

25

The chimeric molecules may contain an immunoglobulin protein or a fragment of an immunoglobulin protein in combination with the polypeptide molecule B29. Likewise, the chimeric molecules may also include immunoglobulins  
30 and immunoglobulin fragments as mentioned above, combined with other polypeptides and polypeptide fragments which are active as described herein. For example, such other polypeptides may include the following:

IgE type receptor proteins (Fc1 RI) on mast cells:  
35 CD3- gamma-  $\delta$  and zeta component proteins of T-cells:

other polypeptides where two amino acids are negatively charged aspartic or glutamic acid groups; the third and fifth amino acids are tyrosine, and the fourth and sixth are leucine or isoleucine.

5

The most preferred chimeric molecules contained herein therefore include molecules and molecular fragments selected from the following compounds: hCD3-gamma, mCD3- $\delta$ , hCD3- $\delta$ , mCD3- $\delta$ , mCD3-zeta, BLVGP 30, hMB1, mMB1, mB29, pFc<sub>1</sub>RI- $\delta$  and rFc<sub>1</sub>RI-B.

10

These molecules typically contain an eighteen amino acid consensus sequence described by Reth, M., NATURE published on March 30, 1989 (page 383), which is incorporated herein by reference.

15

The B-cell derived receptor and related proteins may be prepared alone or in operative association with other molecules or pharmaceutical agents in a form suitable for administration for either diagnostic or therapeutic purposes. The invention thus includes both diagnostic and pharmaceutical compositions including the receptor complex and/or the component proteins, individually and in combination with other diagnostic reagents in the former instance, and in combination with pharmaceutically acceptable carriers, and possibly, other therapeutic agents where coadministration is deemed appropriate or desirable.

20

25

The receptor Ig protein and related proteins possess significant diagnostic and therapeutic capabilities in connection with conditions involving the activity of T- and B-lymphocytes and other humoral immune disease, e.g., autoimmune disease, viral, e.g., HIV and the like, mycobacterial infection, etc.

30  
35

These various diagnostic and therapeutic utilities are predicated on the structures and activities of the receptor and the related proteins. Diagnostic utilities include assays such as immunoassays with labeled  
5 quantities of the receptor, non-immunoglobulin proteins, antibodies, ligand and binding partners thereto, receptor assays, and drug screening assays to evaluate new drugs for the ability to promote or inhibit receptor or non-receptor protein production or activity. The above  
10 assays can also be used to detect the presence or activity of the receptor or non-receptor proteins vis-a-vis invasive stimuli, pathology or injury, the presence or absence of which affect such receptor or non-receptor protein production or activity.

15  
Therapeutic methods and pharmaceutical compositions are based upon the receptor and related proteins, and the vectors described above, which contain sequences coding for materials having the same or antagonistic activity  
20 thereto. Therapeutic methods are generally based on the promotion or inhibition of the activities of the receptor and non-receptor proteins, and thus extends to the treatment of disease or dysfunction attributable to the excess activity or the absence of such activity.

25  
The therapeutic compositions described herein include effective amounts of the receptor molecules and non-immunoglobulin proteins, their agonists, antagonists, antibodies or like drugs, in combination with  
30 pharmaceutically acceptable carriers. Carriers include the pharmacologically inactive ingredients or components used in dosage formulations, e.g., water for injection, tablet adjuvants, capsule fillers and the like. Such compositions can be prepared for a variety of protocols,  
35 including where appropriate, oral and/or parenteral administration. Exact dosages and dosing schedules would be determined by the skilled physician.

Diagnostic and research applications extend generally to the proteins, immunoglobulin as well as non-immunoglobulin, involved in receptor reactivity, and the difference in such reactivity between B- and T-lymphocyte  
5 receptors.

One preferred diagnostic application generally extends to a method for the assessment of immune function. Assays of immune function in animals, including body fluids such  
10 as blood, plasma and urine, tissue samples, and biomolecules such as DNA, will assist in the detection and evaluation of pathology or other systemic dysfunction.

15 The diagnostic and research applications include the performance of several competitive assay protocols, involving the analyte, a ligand and one or more binding partners of interest, where the binding partners are typically selected from the present receptor  
20 immunoglobulin and/or non-immunoglobulin proteins. The binding partners may be generally selected from the group consisting of cells and cellular components having the present receptor, the non-immunoglobulin proteins and other cell proteins.

25 The ligands useful in these applications are generally molecules which are recognized by B-cell receptor IgM proteins. These ligands may be detected either alone or in combination with a second detecting partner such as  
30 avidin.

Standard assays based on the cell components or proteins themselves which employ extract formats may be used. Each assay is capable of being based on enzyme linked  
35 and/or radiolabeled ligands and their binding partners, including the IgM-type receptors and non-receptor proteins disclosed herein. The broad format of the assay

- protocols which are possible within the present invention extends to assays wherein no label is needed for detection. For example, one of the formats contemplates the use of a bound protein-specific receptor. In such
- 5 instance, the analyte would need only to be added to the receptor, and the bound analyte could then be easily detected by a change in the property of the binding partner, such as by a change in the receptor.
- 10 Additionally, assays contemplated herein include assays such as those set forth in the Example, where a specific indicator sequence is contained in the DNA construct. Expression of the protein is thus detected.
- 15 The assays of the invention may follow formats wherein either the ligand or the binding partner, be it a receptor or an antibody, are bound. Likewise, the assays include the use of labels which may be selected from radioactive elements, enzymes and chemicals that
- 20 fluoresce.

The diagnostic applications also include methods for the assessment of immune and other disorders in animals, including humans. The methods comprise assays involving

25 in addition to the analyte, one or more binding partners of the advanced glycosylation endproducts, and one or more ligands.

These diagnostic assay methods broadly comprise the steps

30 of:

- A. preparing at least one biological sample taken from the patient;
- B. preparing at least one corresponding binding partner directed to said sample;
- 35 C. placing a detectible label on a material selected from the group consisting of said sample, a ligand to said binding partner and said binding partner;

D. placing the labeled material from Step C in contact with a material selected from the group consisting of the material from Step C that is not labeled; and

- 5 E. examining the resulting sample of Step D for the extent of binding of said labeled material to said unlabeled material.

Suitable analytes are typically selected from blood,  
10 plasma, urine, cerebrospinal fluid, lymphatic fluid and tissue. Also, the analyte is typically assessed for immune system function.

In the typical competitive assay in accordance with the  
15 present invention, the present receptor immunoglobulin protein, a non-Ig protein or a cellular material bearing such proteins is combined with the analyte and the ligand, and the binding activity of either or both of ligand and analyte to the receptor may then be measured  
20 to determine the extent of binding. In this way, the differences in affinity between the components in the assay serve to characterize reactivity.

In an immunoassay, a control quantity of a binding  
25 partner to the IgM on non-IgM proteins may be prepared and optionally labeled, such as with an enzyme, a compound that fluoresces and/or a radioactive element, and may then be introduced into a tissue or fluid sample of a mammal. After the labeled material or its binding  
30 partner(s) has had an opportunity to react with the sample, the resulting mass may be examined by known techniques, which may vary with the nature of the label.

In general, immunological assays are included which  
35 utilize either a binding partner to the IgM or ligand thereto, optionally labeled with a detectable label, and further optionally including an antibody Ab, labeled with

a detectable label, an antibody  $Ab_2$  labeled with a detectable label, or a chemical conjugate with a binding partner to the protein labeled with a detectable label. The procedures may be summarized by the following  
5 equations wherein the asterisk indicates that the particle is labeled. "BP" in this instance stands for all binding partners of the compound under examination:

- A.  $BP^* + Ab_1 = BP + Ab_1$
- 10 B.  $BP + Ab^* = BPAb_1$
- C.  $BP + Ab_1 + Ab_2^* = BPAb_1Ab_2^*$
- D.  $Carrier^*BP + Ab_1 = Carrier^*BPAb_1$

These general procedures and their application are  
15 familiar to those skilled in the art and are presented herein as illustrative and not restrictive of procedures that may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in the U.S. Patent Nos. 3,654,090 and  
20 3,850,752. Procedure C, a "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043, while procedure D is known as the "double antibody", or "DASP" procedure.

25 A further alternate diagnostic procedure employs multiple labeled compounds in a single solution for simultaneous radioimmuno assay. In this procedure disclosed in U.S. Patent No. 4,762,028 (Olson), a composition may be prepared with two or more analytes in a coordinated  
30 compound having the formula radioisotope-chelator-analyte.

In each instance, IgM or another protein can be used to form complexes with one or more binding partners, and one  
35 member of the complex may be labeled with a detectable label. The fact that a complex has formed and, if

desired, the amount thereof, can be determined by the known applicable detection methods.

One assay format contemplates a bound receptor to which  
5 are added the ligand and the analyte. As used in this example, the receptor can be a compound which binds to the analyte, such as a capture antibody. The resulting substrate is then washed after which detection proceeds by the measurement of the amount of ligand bound to the  
10 receptor. A second format employs bound ligand to which the receptor and the analyte are added. Both of the first two formats are based on a competitive reaction with the analyte, while a third format comprises a direct binding reaction between the analyte and bound receptors. In  
15 this format, bound receptor-specific carrier or substrate is used. The analyte is first added after which the receptor is added, the substrate washed, and the amount of receptor bound to the substrate is measured.

20 More particularly, the present invention includes the following protocol within its scope:

A method for determining immune system function comprising:

A. providing a sample of cells bearing the present  
25 receptor and non-receptor protein(s):

B. inoculating said sample with a known ligand to the receptor, and

C. counting the amount of ligand bound to the receptor.

30

With reference to the use of antibodies as binding partners, it will be seen from the above that a characteristic property of  $Ab_2$  is that it will react with  $Ab_1$ . This is because  $Ab_1$  raised in one mammalian species  
35 has been used in another species as an antigen to raise the antibody  $Ab_2$ . For example,  $Ab_2$  may be raised in goats using rabbit antibodies as antigens.  $Ab_2$  therefore would

be anti-rabbit antibody raised in goats. Where used for purposes of this description, Ab<sub>1</sub> is referred to as the primary antibody and Ab<sub>2</sub> is referred to as a secondary or anti-Ab<sub>1</sub> antibody.

5

The labels most commonly employed the various procedures described herein are radioactive elements, enzymes, chemicals with fluoresce when exposed to ultraviolet light, and other such labels.

10

Suitable radioactive elements may be selected from the group consisting of <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, <sup>36</sup>Cl, <sup>51</sup>Cr, <sup>57</sup>Co, <sup>58</sup>Co, <sup>59</sup>Fe, <sup>90</sup>Y, <sup>125</sup>I, <sup>131</sup>I, and <sup>186</sup>Re. In the instance where a radioactive label, such is prepared with one of the above isotopes is used, known currently available counting procedures may be utilized.

In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, thermometric, amperometric or gasometric techniques known in the art. The enzyme may be conjugated to a protein of interest, binding partners or carrier molecules by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like.

Many indicator enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase,  $\beta$ -glucuronidase,  $\beta$ -D-glucosidase,  $\beta$ -D-galactosidase, urease, glucose, oxidase plus peroxidase, hexokinase plus GPDase, RNase, glucose oxidase plus alkaline phosphatase, NAD oxidoreductase plus luciferase, phosphofructokinase plus phosphoenol pyruvate carboxylase, aspartate aminotransferase plus phosphoenol pyruvate decarboxylase, and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are

referred to by way of example for their disclosure of alternative labeling material and methods.

A number of fluorescent materials are also known and an  
5 be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

10

The assays described above may be prepared or used in the form of test kits. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a  
15 label to a binding partner, such as the IgM protein, a receptor or ligand as listed herein; and one or more additional immunochemical reagents, at least one of which is a free or immobilized ligand, capable either of binding with the labeled component, its binding partner,  
20 one of the components to be determined or their binding partner(s).

Commercial test kits suitable for use by a medical specialist may also be prepared. In accordance with the  
25 testing techniques discussed above, one class of such kits contains at least a labeled binding partner as state above, and directions, of course, depending upon the method selected, e.g. "competitive", "sandwich", "DASP" and the like. The kits may also contain peripheral  
30 reagents such as buffers, stabilizers, etc.

The corresponding diagnostic test kit may comprise:

(a) a known amount of a binding partner as described above, or a ligand thereof, generally bound to  
35 a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc, (or their binding partners) one of each;

- (b) if necessary, other reagents; and
- (c) directions for use of said test kits.

Another variation, of the test kit comprises;

- 5 (a) a labeled component which has been obtained by coupling the above binding partner to a detectable label;
  - (b) one or more additional immunochemical reagents to which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the
  - 10 group consisting of:
    - (i) a ligand capable of binding with the labeled component (a);
    - (ii) a ligand capable of binding with a binding partner of the labeled component (a);
    - 15 (iii) a ligand capable of binding with at least one of the components to be determined; and
    - (iv) a ligand capable of binding with at least one of the binding partners of at least one of the components to be determined; and
  - 20 (c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the compound presently assessed and the binding partner.
- 25 The discovery of a method of overcoming T-cell dependency on the presence or activity of MHC makes possible new therapeutic modalities, such as for diseases where MHC is absent or inactive. T-lymphocytes taken from a patient can be modified as described herein and reinfused into
- 30 the patient to restore T-cell function in an immunocompromised patient. By transfecting the T-lymphocytes with the gene for B-cell derived IgM and related proteins, a therapy can be designed to improve T-cell function in the patient in need of such treatment.
- 35 Since T-lymphocytes are the effectors of a large number of cellular immune responses, including but not limited

to tumor rejection and protection from infection (viral, parasitic and mycobacterial) such diseases can be treated in accordance with the teachings contained herein. The nature of the antigens normally recognized by these cells is tailored to the particular individual, because T-lymphocytes recognize a combination of antigen plus cell surface MHC molecule(s). Since MHC is highly polymorphic in humans, naturally occurring T-cells that recognize any of the above mentioned challenges are not useful if transferred between individuals; the T-cells from one individual are unable to recognize antigen in other individuals because the endogenous MHC is not perceived by the T-lymphocytes which are infused. For example, T-cells from individual Y only recognize tumor cells in individual Y. When transferred to individual X, the T-cells do not recognize tumor cells because of the differences in MHC between individuals X and Y. This is the case even between like tumor types.

The invention described herein encompasses a method of changing the nature of antigen recognition by T-cells, such that these cells will "see" antigen without the presence of MHC. T-cells in accordance herewith, can be transfected with monoclonal antibodies which recognize a given antigen. In this manner, two individuals with the same tumor or tumor type can be treated by administering to each an effective amount of the T-cells described above, which recognize the particular tumor or tumor type. This overcomes or circumvents the differences in MHC between individuals. Hence, by introducing specific antibody genes to target the T-cells to specific antigens in different individuals, the same composition can be used in all patients with a given disease. It is therefore clear that treating T-lymphocytes as described herein with B-cell derived IgM and B29, and a monoclonal antibody which recognizes and binds to a specific antigen

can impart recognition of the antigen to T-cells without the presence or activity of MHC.

Specific monoclonal antibodies now used to target  
5 radiolabeled compounds or toxins to tumors can be included as donors of the antibody specificity to be transferred into T-cells.

The techniques for transfection of T-cells described  
10 above, and the infusion thereof into patients can also be used to produce a T-cell line having the desired non-MHC specificity for purposes of secreting selected lymphokines and delivering such lymphokines to specific sites of activity within the mammal. Such T-cells with  
15 this specificity can then be used to deliver normal T-cell lymphokines, such as IL-2, IL-4 and interferons to the specific areas of involvement. These agents, which are known to have potent pharmacological effects, would act locally in high local concentrations as opposed to  
20 systemic delivery with problematic side effects. Particular monoclonal antibodies can thus be selected which recognize the tumor or tumor type to be treated. In addition, the T-cells themselves are cytotoxic and kill tumors directly.

25

In a large number of viral infections, T-cells play a role in combating infection. One potential target is HIV. In this case, T-cells can be engineered to be specific for the virus by transferring antibodies that  
30 are specific for the portion of gp120 that binds to CD4. These recipient T-cells would preferably have the CD4 protein component thereof deleted, such as by a gene knockout technique so that these modified cells would not in and of themselves be susceptible to infection.

35

Likewise there are a number of mycobacterial organisms which are at least in part resistant to chemotherapeutic

agents. Examples include the tuberculosis and leprosy mycobacteria. Monoclonal antibodies specific for these mycobacteria could be transfected into T-cells, which are the key mediators for successful immune responses in these diseases. The T-cells would then be introduced into the patient, to in turn deliver the required lymphokines and the T-cell cytotoxicity at the site of infection.

- 10 A preferred embodiment of the invention is further illustrated in the Example. However, the scope of the claims is not to be limited thereby.

#### EXAMPLE

15

##### Plasmid Construction

The Spleen Focus Forming Virus, Long Term Repeat (SFFV LTR) promoter from pFNeo (Saito et al., 1987) was combined with the BamH1 to EcoR1 fragment of the human growth hormone (hGH) to produce an SFFV cDNA expression vector (p463). The hGH sequences and polyadenylation signals were added to increase mRNA stability for cDNA expression. MB1 and B29 cDNAs obtained by polymerase chain reaction (PCR) were sequenced and cloned into the polylinker of p463 to obtain the p466-B29 and the p467-MB1 expression vectors. The EcoR1 fragment of the MB1 expression clone was then transferred to the EcoR1 site of pSV2His (Hartman and Mulligan, 1988) to produce a plasmid that carries both an MB1 expression vector and His resistance, p474-MB1.

The IgM heavy chain minigene was composed of the V region of S107 (Crews et al., 1981) ligated to the Hind3 to BamH1 fragment of a human IgM constant region gene that has been modified to produce only the membrane bound form of IgM protein (Danner and Leder, 1985). The IgM minigene was cloned just 3' of the SFFV LTR in pFneo in

the Sall to BamH1 site to make plasmid p459. The kappa minigene was composed of the S107 light chain V region (Kwan et al., 1981) ligated to the EcoR1 to BamH1 fragment of human kappa light chain constant region (Hieter et al., 1982) and the promoter was the EcoR1 to BamH1 fragment of the SFFV LTR. The heavy and light chains were combined in a single plasmid by cloning the light chain minigene into the unique BamH1 site of p459 to produce p468, a plasmid that carries the PC specific heavy and light chains, and neomycin resistance genes.

#### Cells Lines

Jurkat cells were grown in RPMI 1640 supplemented with 10% bovine calf serum, 50U/ml penicillin, 50mg/ml streptomycin and 2mM L-glutamine (R10). Cells were transfected with linear plasmid DNA by electroporation (Potter et al., 1984), and selection was carried out in R10 with 0.7 mg/ml of G418 (Gibco), and/or 5mM L-Histidinol (Sigma). Resistant cell lines were stained with 10mg/ml fluorescein labeled goat anti-human IgM (Southern Biotechnology) and positive cells sorted on a Facstar Plus (Becton-Dickinson). Analysis of surface staining was performed with the same antibody using a Facscan (Becton-Dickinson).

25

#### Antibodies

Monoclonal anti-human IgM, DA4.4 (Maruyama et al., 1985), and anti-human CD-3, OKT 3 (Kung et al., 1979), were purified from either ascites or tissue culture media by precipitation with ammonium sulfate and chromatography on protein-A sepharose. Goat anti-human IgM either labeled with fluorescein or unlabeled, IgG1 monoclonal isotype control antibody, and goat anti-mouse IgG were from Southern Biotechnology.

35

### Northern Analysis

RNA was prepared as described (Chirgwin et al., 1979). Following electrophoresis, and transfer blots were hybridized with either B29 cDNA or MB1 anti-sense RNA as described (Sambrook et al., 1989).

### Iodinations, Immunoaffinity Purification and Protein Electrophoresis

3-6X10<sup>7</sup> viable cells were labeled with 3-4 mCi of Na[<sup>125</sup>I] and lactoperoxidase/glucose oxidase as described (Hubard and Cohn, 1972) and solubilized in 1% digitonin, 100 mM NaCl, 50 mM Tris HCl (pH 6.8) and 1 mM phenylmethylsulfonyl fluoride at 4°C for 30 minutes. Insoluble material was separated by centrifugation and the supernatant was incubated with phosphorylcholine beads for 4-5 hours. The beads were washed three times with 0.1% digitonin, 100 mM NaCl and 50 mM Tris HCl (pH 6.8) for five minutes. Elution of IgM was carried out by incubating the beads for 1 hour with wash buffer supplemented with 20 mM phosphorylcholine. Protein samples were analyzed on 8-12% SDS-PAGE on gradient gels under reducing conditions. The protein bands were visualized by silver staining. A Phosphor Imager (Molecular Dynamics) was used to visualize <sup>125</sup>I labeled proteins.

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### Calcium Flux Measurements

Cells were resuspended at 5X10<sup>6</sup>/ml in phosphate buffered saline (PBS) supplemented with 5.6 mM glucose, 0.025% BSA, 20 mM HEPES (pH 7.0), 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> (loading buffer) and 3 mg/ml Fura-2AM (Sigma), and incubated at 37°C for 30 minutes followed by three washes with loading buffer. The Fura-2 loaded cells were then resuspended in loading buffer at a concentration of 1X10<sup>6</sup>/ml and 2ml loaded into the cuvette of a SPF-500C spectrofluorometer (SLM AMINCO Instruments Inc.). The excitation wavelength was 335nm and emission was measured at 510nm. Calcium

concentration was calculated as described (Gryniewicz et al., 1985).

#### Synthesis of PC-albumin and PC-sepharose

5 Para-diazoniumphenylphosphorylcholine (DPPC) was synthesized (Chesebro and Metzger, 1972) and BSA dissolved in PBS was added and incubated overnight. Modified BSA (PC-albumin) was separated from unreacted DPPC by column chromatography on Sephadex G25 in PBS.

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For the synthesis of PC-beads, the dipeptide alanyltyrosine was coupled to CNBr-activated Sepharose (Pharmacia). The beads were washed in PBS and freshly synthesized DPPC was added, incubated overnight, washed with water and stored at 15 4°C.

#### IL-2 Bioassay

Cells to be tested for IL-2 production were resuspended at  $1.2 \times 10^6$ /ml in tissue culture media and used directly or  
20 pretreated with either 10 ug/ml anti-CD3, 20 ug/ml of DA4.4 monoclonal anti-human IgM, or a 10ug /ml of an IgG1 monoclonal isotype control antibody for 30 min at 4°C. Unbound antibody was removed by extensive washing with PBS.  $2 \times 10^4$  cells were then plated in the wells of a 96  
25 well tissue culture plate pre-coated with either 10 mg/ml goat anti-mouse IgG (Southern Biotechnology), 10 mg/ml PC-Albumin, 10 mg/ml bovine serum albumin or nothing. Phobol myristate acetate (PMA) was added at a final concentration of 10 ng/ml. Supernatants were harvested  
30 after 24 hours and bioassays were performed using the CTLL-2.20 IL-2 dependent cell line as described (Gillis et al., 1978).

#### Inositol Phosphate Measurement

35 Cells were loaded for 4 hours with  $^3\text{H}$ -myoinositol at 15uCi/ml, and  $3-4 \times 10^6$  cells/ml in inositol free RPMI 1640 supplemented with 10% dialyzed bovine calf serum. Excess

<sup>3</sup>H myoinositol was removed by washing in PBS. The loaded cells were then resuspended at 2X10<sup>6</sup>/ml in HEPES-buffered saline supplemented with 10mM LiCl and equilibrated for 15 min at 37°C. The cells were sequentially stimulated with 1 ug/ml of either anti-CD3, or DA4.4 monoclonal anti-human IgM or the IgG1 isotype control followed after 10 seconds by 10 ug/ml of goat anti-mouse IgG crosslinking reagent. The incubation was terminated after 3 min by the addition of 8 ml of 2:1 methanol:chloroform, and the extracted material was subjected to chromatography on 1ml columns of Dowex AG-1-8 (Bio-Rad). Inositol phosphates (IP) were eluted with 0.1 M formic acid and increasing concentrations of ammonium formate, (0.2 M for inositol phosphate, 0.4 M for IP<sub>2</sub>, 0.8 M for IP<sub>3</sub>, and 1 M for IP<sub>4</sub>). The eluted radioactivity was quantitated by liquid scintillation counting.

- Numerous patent and journal publications cited throughout the specification for procedures and processes which are used or referred to in the specification. These publications are hereby incorporated by reference.
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This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

30

WHAT IS CLAIMED:

- 1 1. A DNA construct comprised of the expression  
2 sequences, fragments or derivatives thereof, which code  
3 for both an IgM immunoglobulin and the protein B29.
- 1 2. A construct in accordance with claim 1 wherein the  
2 immunoglobulin is comprised of a polypeptide of the B-  
3 lymphocyte receptor.
- 1 3. A construct in accordance with claim 1 wherein the  
2 immunoglobulin is a fragment of IgM.
- 1 4. A construct in accordance with claim 3 wherein the  
2 fragment is a light chain fragment of IgM.
- 1 5. A construct in accordance with claim 3 wherein the  
2 fragment is a heavy chain fragment.
- 1 6. A construct in accordance with claim 1 in the  
2 substantial absence of a sequence, fragment or derivative  
3 thereof which codes on expression for the protein MB1.
- 1 7. An RNA molecule which codes on expression for both an  
2 IgM immunoglobulin and the protein B29.
- 1 8. An RNA molecule in accordance with claim 7 wherein  
2 the immunoglobulin is comprised of a polypeptide of the  
3 B-lymphocyte receptor.
- 1 9. An RNA molecule in accordance with claim 7 wherein  
2 the immunoglobulin is a fragment of an IgM polypeptide  
3 receptor or signaling molecule.
- 1 10. An RNA molecule in accordance with claim 9 wherein  
2 the fragment is a light chain fragment of the IgM  
3 receptor.

1 11. An RNA molecule in accordance with claim 9 wherein  
2 the fragment is a heavy chain fragment.

1 12. An antibody raised to an IgM receptor molecule  
2 present on or derived from T-lymphocytes.

1 13. An antibody in accordance with claim 12 wherein the  
2 IgM receptor molecule present on T-lymphocytes is in  
3 combination with the polypeptide B29.

1 14. An antibody in accordance with claim 12 wherein the  
2 IgM receptor molecule present on T-lymphocytes is in  
3 combination with the polypeptides B29 and MB1.

1 15. An antibody in accordance with claim 12, 13 or 14  
2 which is polyclonal.

1 16. An antibody in accordance with claim 12, 13 or 14  
2 which is monoclonal.

1 17. A chimeric molecule which is comprised of an  
2 immunoglobulin protein or a fragment thereof and the  
3 polypeptide B29 or a fragment thereof.

1 18. A chimeric molecule which is comprised of an  
2 immunoglobulin protein or a fragment thereof, and a  
3 polypeptide or fragment thereof selected from the group  
4 consisting of:

5 a) IgE type receptor proteins (Fc<sub>γ</sub>RI-β and gamma)  
6 which can be derived from mast cells;

7 b) CD3-gamma, -δ and zeta component proteins which  
8 can be derived from T-cells;

9 c) other molecules and molecular fragments which  
10 can be derived from hCD3-gamma, mCD3-gamma, hCD3-δ, mCD3-  
11 δ, mCD3-zeta, BLVgp30, hMB1, mMB1, mB29, pFc<sub>γ</sub>RI-gamma and  
12 rFc<sub>γ</sub>RI-β, and

13           d) other polypeptides or fragments thereof, where  
14 the first two amino acids are aspartic or glutamic acid  
15 groups; the third and fifth amino acids are tyrosine, and  
16 the fourth and sixth amino acids are leucine or  
17 isoleucine groups.

1   19. A chimeric antibody comprised of

2           (a) a heavy chain containing a human membrane-  
3 anchored IgM constant region and a phosphorylcholine  
4 specific heavy chain variable region, and

5           (b) a light chain containing a human kappa constant  
6 region and a kappa variable region.

1   20. An antiserum comprised of antibodies in accordance  
2 with any one of claims 12, 13, 14, 17 or 18 in  
3 combination with a pharmaceutically acceptable carrier.

1   21. A method of producing an antiserum in accordance  
2 with claim 20, comprising administering to substantially  
3 immunocompetent mammal an antibody-raising, effective  
4 amount of a T-lymphocyte receptor derived immunoglobulin  
5 protein, and  
6           harvesting the antiserum from said mammal.

1   22. A method in accordance with claim 21 wherein the  
2 immunoglobulin protein is administered to said mammal in  
3 combination with the polypeptide B29 or a fragment  
4 thereof.

1   23. An expression vector containing the DNA construct of  
2 any one of claims 1 through 6.

1   24. An expression vector containing the RNA molecule of  
2 any one of claims 7 through 11.

1   25. An expression vector containing a gene which codes  
2 on expression for an IgM heavy chain or fragment thereof

3 containing a human membrane anchored IgM constant region.

1 26. An expression vector in accordance with claim 25  
2 further containing a gene which codes on expression for  
3 an IgM heavy chain phosphorylcholine-specific variable  
4 region.

1 27. An expression vector in accordance with claim 25  
2 further containing a gene which codes on expression for  
3 an IgM light chain containing a human kappa constant  
4 region.

1 28. An expression vector in accordance with claim 25  
2 further comprised of a gene which codes on expression for  
3 an IgM light chain kappa variable region.

1 29. An expression vector containing genes which code on  
2 expression for a chimeric antibody in accordance with  
3 claim 19.

1 30. A unicellular host transformed with the DNA  
2 construct of any one of claims 1 through 6.

1 31. A unicellular host transformed with the RNA molecule  
2 of any one of claims 7 through 11.

1 32. A unicellular host in accordance with claim 30  
2 further transformed with an antibody.

1 33. A unicellular host in accordance with claim 31  
2 further transformed with an antibody.

1 34. A unicellular host in accordance with claim 32  
2 wherein the antibody is a monoclonal antibody.

1 35. A unicellular host in accordance with claim 34  
2 wherein the monoclonal antibody recognizes a tumor cell  
3 or component thereof.

1 36. A unicellular host in accordance with claim 34  
2 wherein the monoclonal antibody recognizes a virus or  
3 part thereof.

1 37. A unicellular host in accordance with claim 36  
2 wherein the monoclonal antibody recognizes at least a  
3 portion of the gp120 site which binds to the CD4  
4 receptor.

1 38. A recombinant virus containing the DNA construct of  
2 any one of claims 1-6.

1 39. A recombinant virus containing the RNA molecule of  
2 any one of claims 7-11.

1 40. A recombinant virus containing a gene or a  
2 derivative thereof, which codes on expression for the IgM  
3 heavy chain or fragment thereof containing a human  
4 membrane anchored IgM constant region.

1 41. A recombinant virus in accordance with claim 40  
2 containing a derivative of the gene which codes on  
3 expression for the IgM heavy chain or fragment thereof  
4 containing a human membrane anchored IgM constant region  
5 in the form of an RNA sequence.

1 42. A T-lymphocyte, mast cell or macrophage transformed  
2 with a DNA construct in accordance with any one of claims  
3 1-6 or an RNA molecule in accordance with any one of  
4 claims 7-11.

1 43. A T-lymphocyte, mast cell or macrophage in  
2 accordance with claim 42 which is further modified by

3 deleting the gene from the genome contained therein which  
4 codes on expression for the CD4 receptor.

1 44. A T-lymphocyte, mast cell or macrophage in  
2 accordance with claim 42 further transformed with an  
3 antibody.

1 45. A T-lymphocyte, mast cell or macrophage in  
2 accordance with claim 43 further transformed with an  
3 antibody which binds to the HIV virus or a component  
4 thereof.

1 46. A T-lymphocyte, mast cell or macrophage in  
2 accordance with claim 44 wherein the antibody is a  
3 monoclonal antibody which binds to a tumor, a  
4 mycobacterial organism or a virus, or a component of such  
5 tumor, mycobacterial organism or virus.

1 47. A method of modifying T-lymphocyte immunity  
2 comprising transfecting T-lymphocytes with genes which  
3 code on expression for both a B-lymphocyte derived  
4 immunoglobulin and the protein B29.

1 48. A method in accordance with claim 47 further  
2 comprising transfecting said T-lymphocytes with a gene  
3 which codes on expression for the protein MB1.

1 49. A method in accordance with claim 47 further  
2 comprising transfecting the T-lymphocytes with an  
3 antibody.

1 50. A method in accordance with claim 49 wherein the  
2 antibody is a monoclonal antibody raised to a tumor,  
3 virus, parasite or mycobacteria, or a component of said  
4 tumor, virus, parasite or mycobacteria.

1 51. A method for screening a compound for  
2 immunomodulatory activity comprising:  
3 combining the compound with T-lymphocytes modified  
4 in accordance with claim 47, 48 or 49;  
5 measuring calcium flux, phosphoinositol turnover or  
6 lymphokine expression in said T-lymphocytes in response  
7 to an antigen challenge, and  
8 comparing said calcium flux, phosphoinositol  
9 turnover or lymphokine expression to a standard.

1 52. A method of enhancing B-lymphocyte derived IgM  
2 immunoreactivity on the cell surface of T-lymphocytes,  
3 mast cells or macrophage cells transformed with the DNA  
4 construct of any one of claims 1-6, or the RNA molecule  
5 of any one of claims 7-11, comprising treating said T-  
6 lymphocytes, mast cells or macrophage cells with the  
7 protein MB1 or transfecting said T-lymphocytes with a  
8 gene or a derivative of said gene which codes on  
9 expression for the protein MB1.

1 53. A method of treating an immune system disorder, a  
2 tumor, or a mycobacterial, parasitic or viral infection  
3 in a mammalian patient in need of such treatment,  
4 comprising administering to the mammal an effective  
5 amount of the T-lymphocytes, mast cells or macrophage  
6 cells of claim 42.

1 54. A method of treating an immune system disorder, a  
2 tumor, or a mycobacterial, parasite or viral infection in  
3 a mammalian patient in need of such treatment comprising  
4 administering to the mammal an effective amount of the T-  
5 lymphocytes, mast cells or macrophage cells of claim 44.

1 55. A method of treating a viral infection in a  
2 mammalian patient in need of such treatment, comprising  
3 administering to the mammal an effective amount of T-

4 lymphocytes, mast cells or macrophage cells modified in  
5 accordance with claim 49.

1 56. A method of treating HIV infection in a mammalian  
2 patient in need of such treatment, comprising  
3 administering to said patient an effective amount of the  
4 T-lymphocytes, mast cells or macrophage cells of claim  
5 43.

1 57. A method of treating HIV infection in a mammalian  
2 patient in need of such treatment, comprising  
3 administering to said patient an effective amount of the  
4 T-lymphocytes, mast cells or macrophage cells of claim  
5 45.

1 58. A method of administering a lymphokine to the site  
2 of a tumor or infection in a mammalian patient in need of  
3 such treatment, comprising administering to said patient  
4 an effective amount of T-lymphocytes, mast cells or  
5 macrophage cells transfected with a DNA sequence coding  
6 on expression for both a B-lymphocyte derived IgM protein  
7 and the protein B29, and further transfected with an  
8 antibody raised to the tumor or tumor type, to the  
9 infectious organism or to a fragment of such tumor or  
10 organism,

11 said T-lymphocytes being essentially non-dependent  
12 on MHC presence or APC activity and being suitable for  
13 expressing said lymphokine upon binding to said tumor or  
14 infectious organism.

1 59. A method of detecting the presence, level or  
2 activity of a tumor tissue in a sample, comprising:  
3 combining the sample with a quantity of the T-  
4 lymphocytes, mast cells or macrophage cells transformed  
5 with genes which code on expression for both a B-  
6 lymphocyte derived IgM protein and the protein B29, and a

7 gene which codes on expression for an antibody which  
8 recognizes and binds to tumor tissue;  
9 measuring the amount of lymphokine expressed by said  
10 T-lymphocytes, mast cells or macrophage cells; and  
11 comparing the amount of lymphokine expressed to a  
12 standard.

1 60. A method of detecting the presence, quantity or  
2 activity of infectious organisms in a sample, comprising:  
3 combining the sample with T-lymphocytes, mast cells  
4 or macrophage cells transformed with a gene which codes  
5 on expression for a B-lymphocyte derived IgM receptor  
6 protein, another gene which codes on expression for the  
7 protein B29 and an antibody which recognizes infectious  
8 organisms:  
9 measuring the amount of lymphokine expressed by said  
10 T-lymphocytes, mast cells or macrophage cells, and  
11 comparing the amount of lymphokine expressed to a  
12 standard.

1 61. A kit for performing the method of claim 59 or 60  
2 comprising an amount of T-lymphocytes, mast cells or  
3 macrophage cells transfected as described therein, a  
4 labelled component which detects lymphokine and  
5 directions for conducting such method.

1 62. A pharmaceutical composition comprised of a DNA  
2 construct in accordance with any one of claims 1-6 or an  
3 RNA molecule in accordance with any one of claims 7-11 in  
4 combination with a pharmaceutically acceptable carrier.

1 63. A pharmaceutical composition comprised of an  
2 antibody in accordance with claim 19 in combination with  
3 a pharmaceutically acceptable carrier.

1 64. A pharmaceutical composition comprised of a  
2 unicellular host in accordance with claim 30 in  
3 combination with a pharmaceutically acceptable carrier.

1 65. A pharmaceutical composition comprised of a  
2 unicellular host in accordance with claim 31 in  
3 combination with a pharmaceutically acceptable carrier.

1 66. A pharmaceutical composition comprised of a  
2 unicellular host in accordance with claim 32 in  
3 combination with a pharmaceutically acceptable carrier.

1 67. A pharmaceutical composition comprised of a  
2 unicellular host in accordance with claim 33 in  
3 combination with a pharmaceutically acceptable carrier.

1 68. A pharmaceutical composition containing a  
2 recombinant virus according to claim 38 in combination  
3 with a pharmaceutically acceptable carrier.

1 69. A pharmaceutical composition containing a  
2 recombinant virus according to claim 39 in combination  
3 with a pharmaceutically acceptable carrier.

1 70. A pharmaceutical composition containing a  
2 recombinant virus according to claim 40 in combination  
3 with a pharmaceutically acceptable carrier.

1 71. A pharmaceutical composition containing a  
2 recombinant virus according to claim 41 in combination  
3 with a pharmaceutically acceptable carrier.

1 72. A pharmaceutical composition containing T-  
2 lymphocytes, mast cells or macrophage cells in accordance  
3 with claim 42 in combination with a pharmaceutically  
4 acceptable carrier.

1 73. A pharmaceutical composition containing T-  
2 lymphocytes, mast cells or macrophage cells in accordance  
3 with claim 43 in combination with a pharmaceutically  
4 acceptable carrier.

1 74. A pharmaceutical composition containing a  
2 recombinant virus according to claim 44 in combination  
3 with a pharmaceutically acceptable carrier.

1 75. A pharmaceutical composition containing a  
2 recombinant virus according to claim 45 in combination  
3 with a pharmaceutically acceptable carrier.

1 76. A pharmaceutical composition containing a  
2 recombinant virus according to claim 46 in combination  
3 with a pharmaceutically acceptable carrier.

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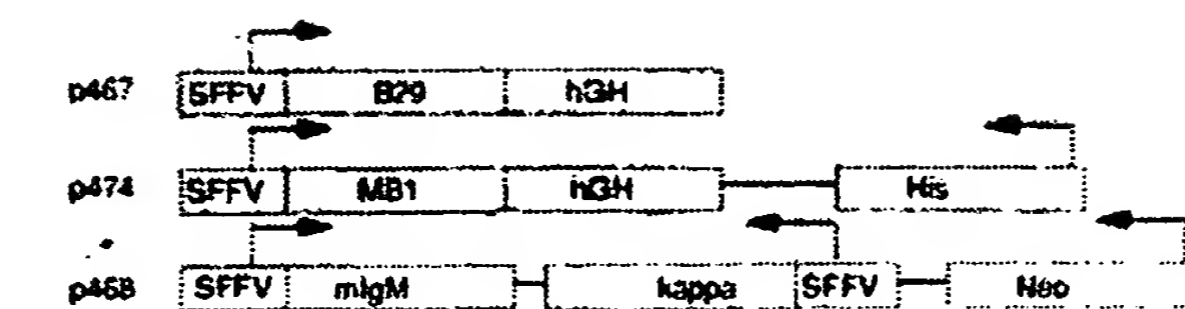


FIG. IA

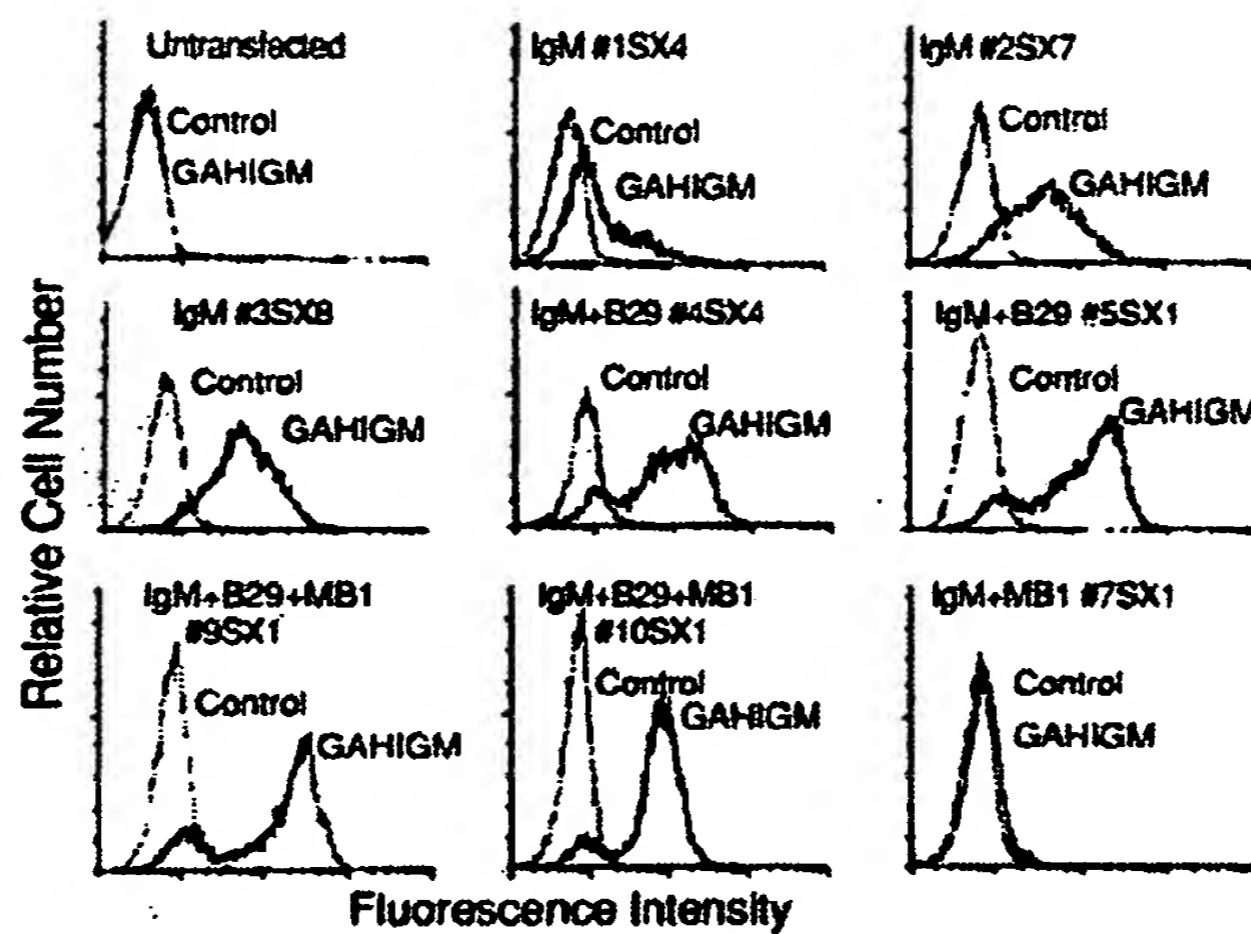


FIG. IB

SUBSTITUTE SHEET

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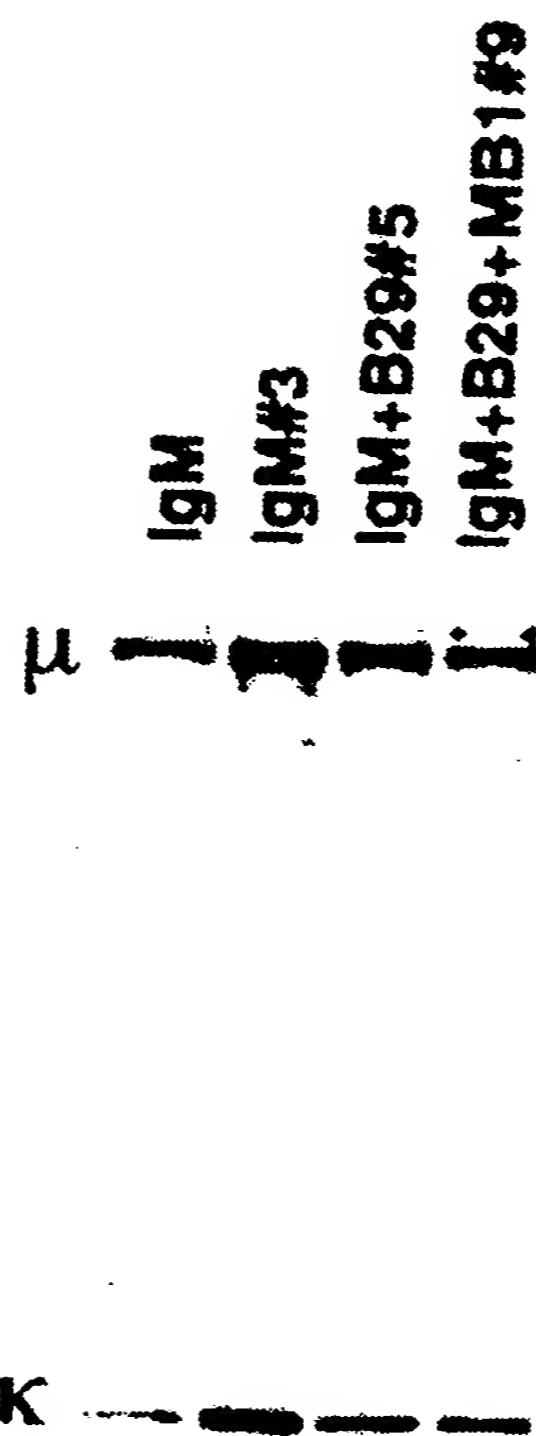


FIG. 2A

SUBSTITUTE SHEET

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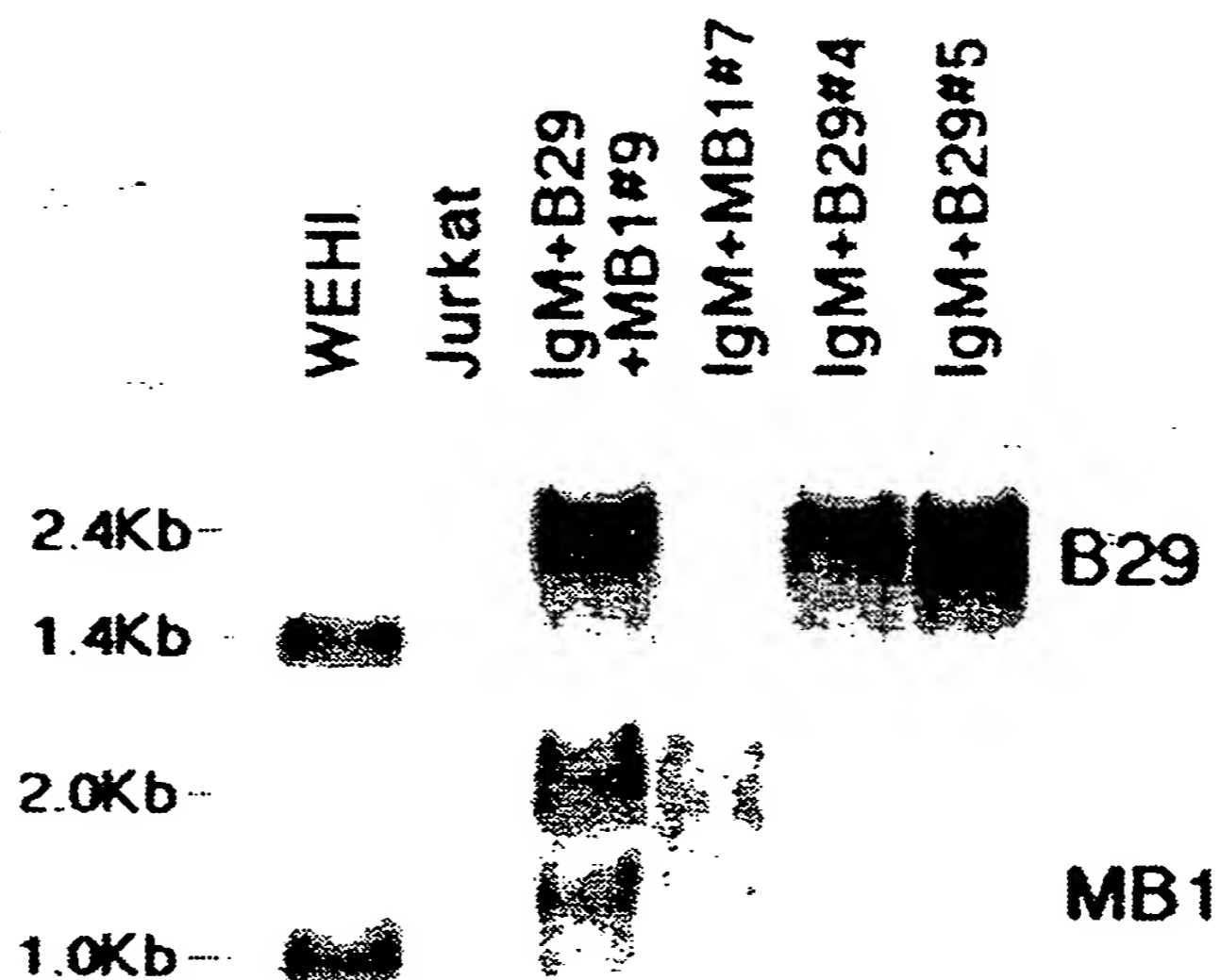


FIG. 2B

SUBSTITUTE SHEET

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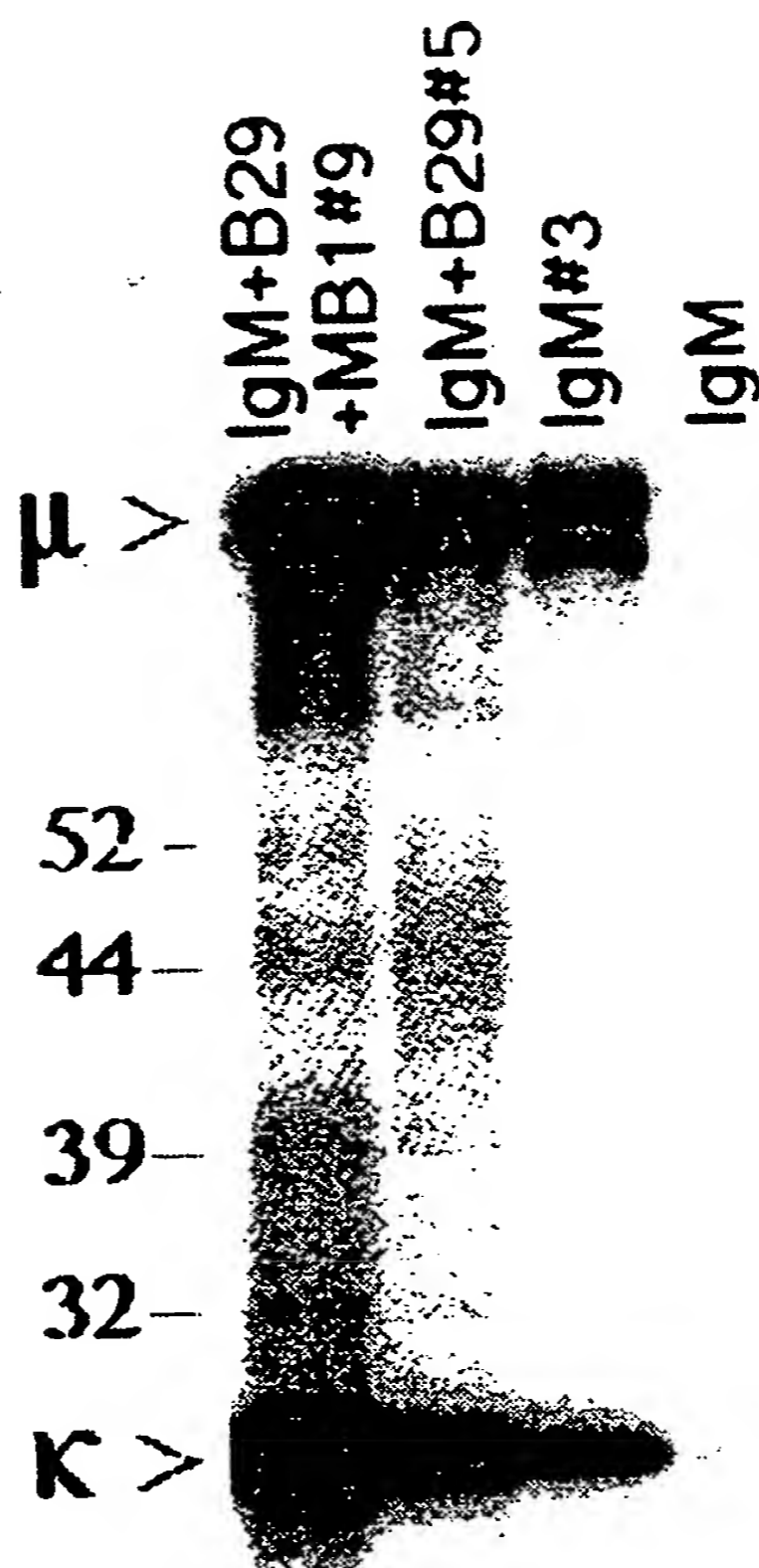


FIG. 3

SUBSTITUTE SHEET

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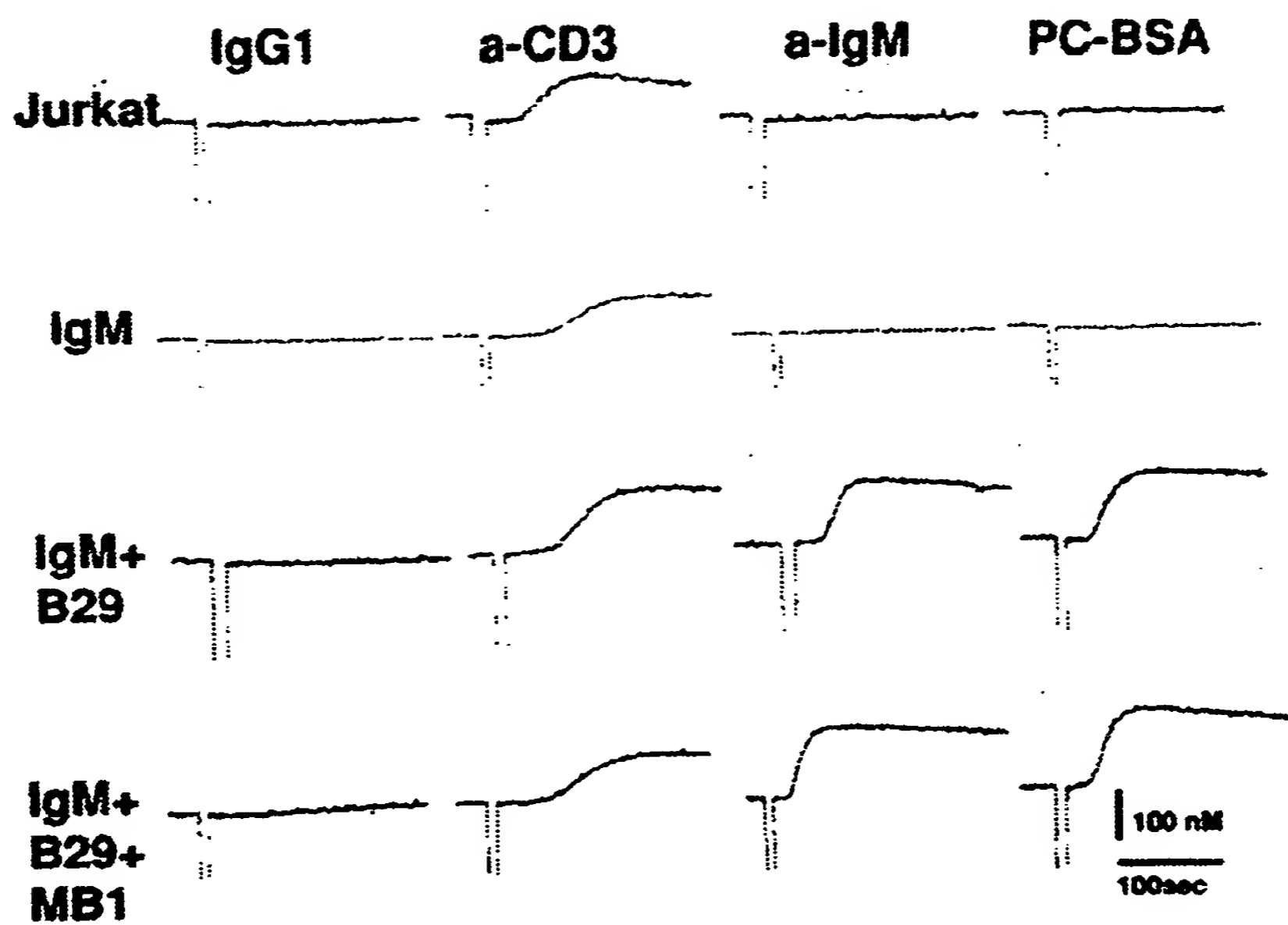


FIG. 4

SUBSTITUTE SHEET

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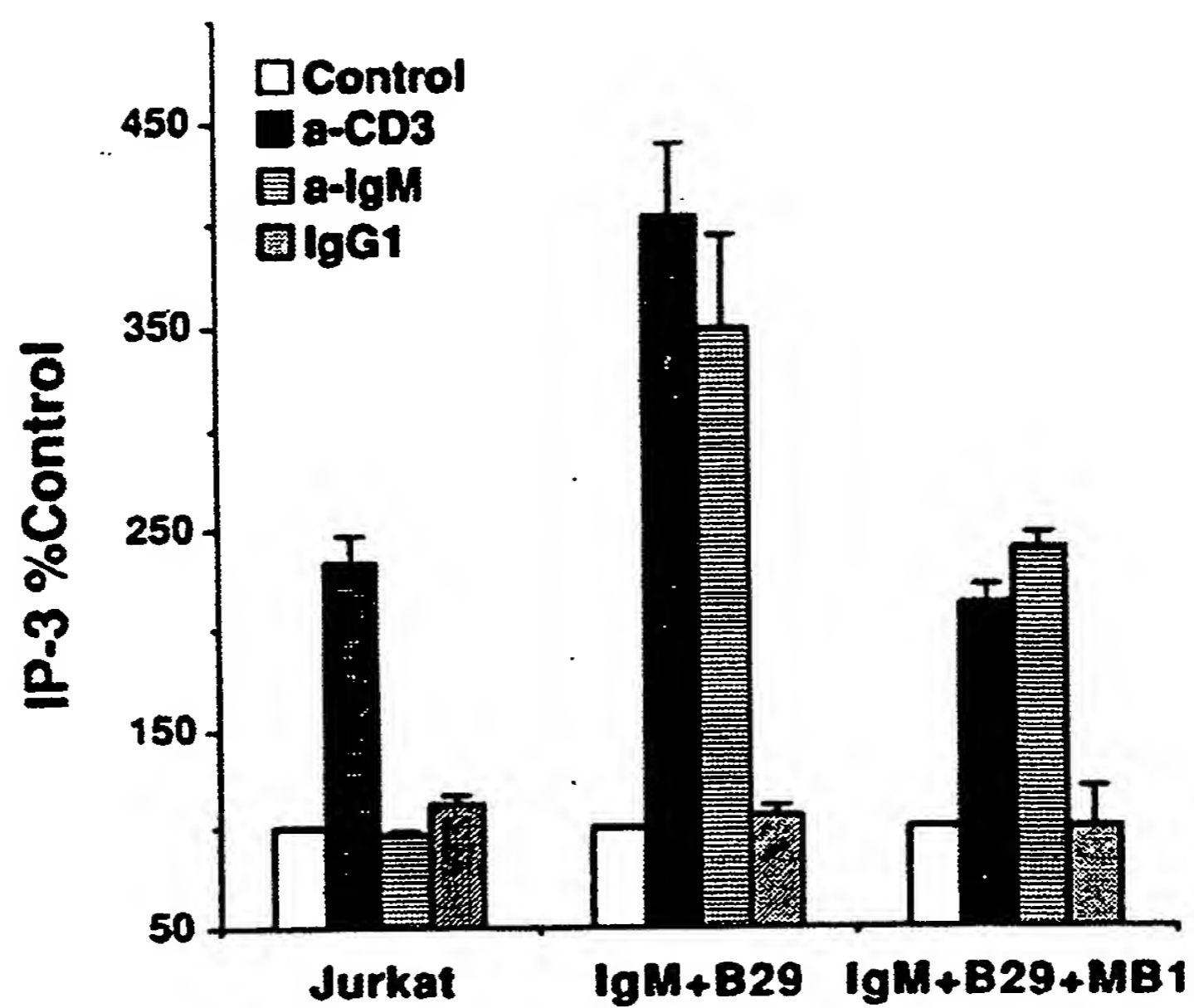


FIG. 5

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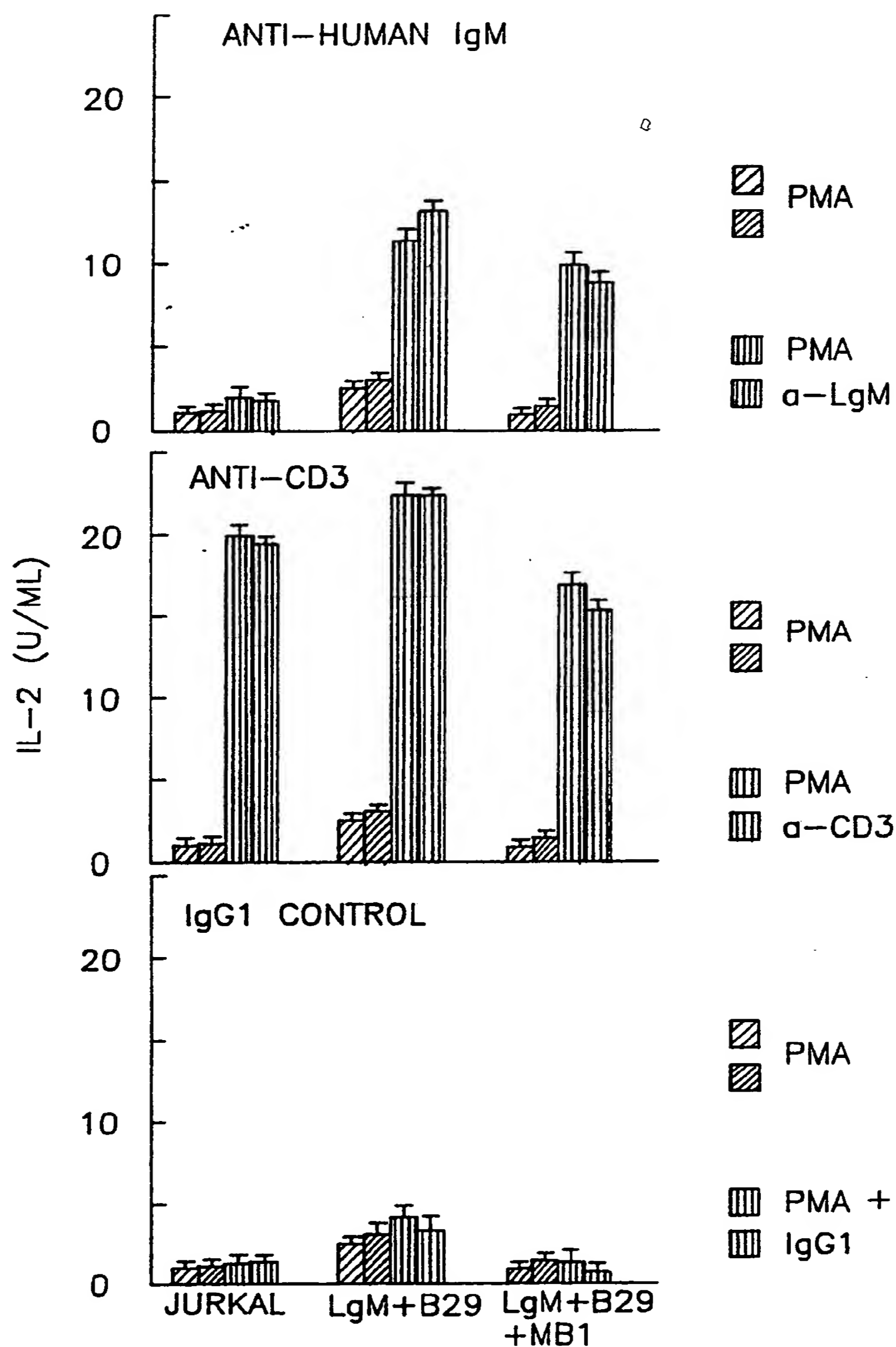


FIG. 6

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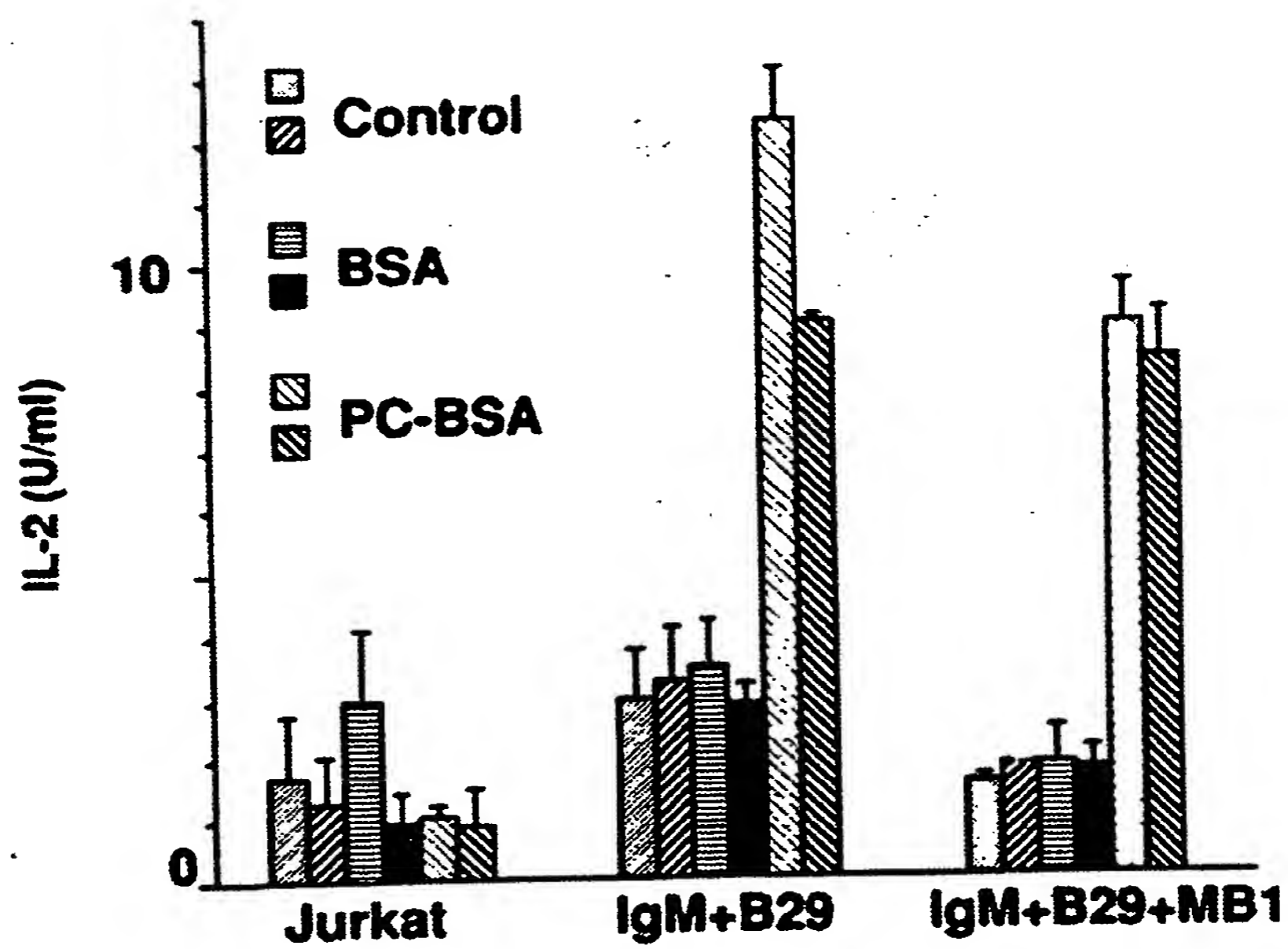


FIG. 7

SUBSTITUTE SHEET

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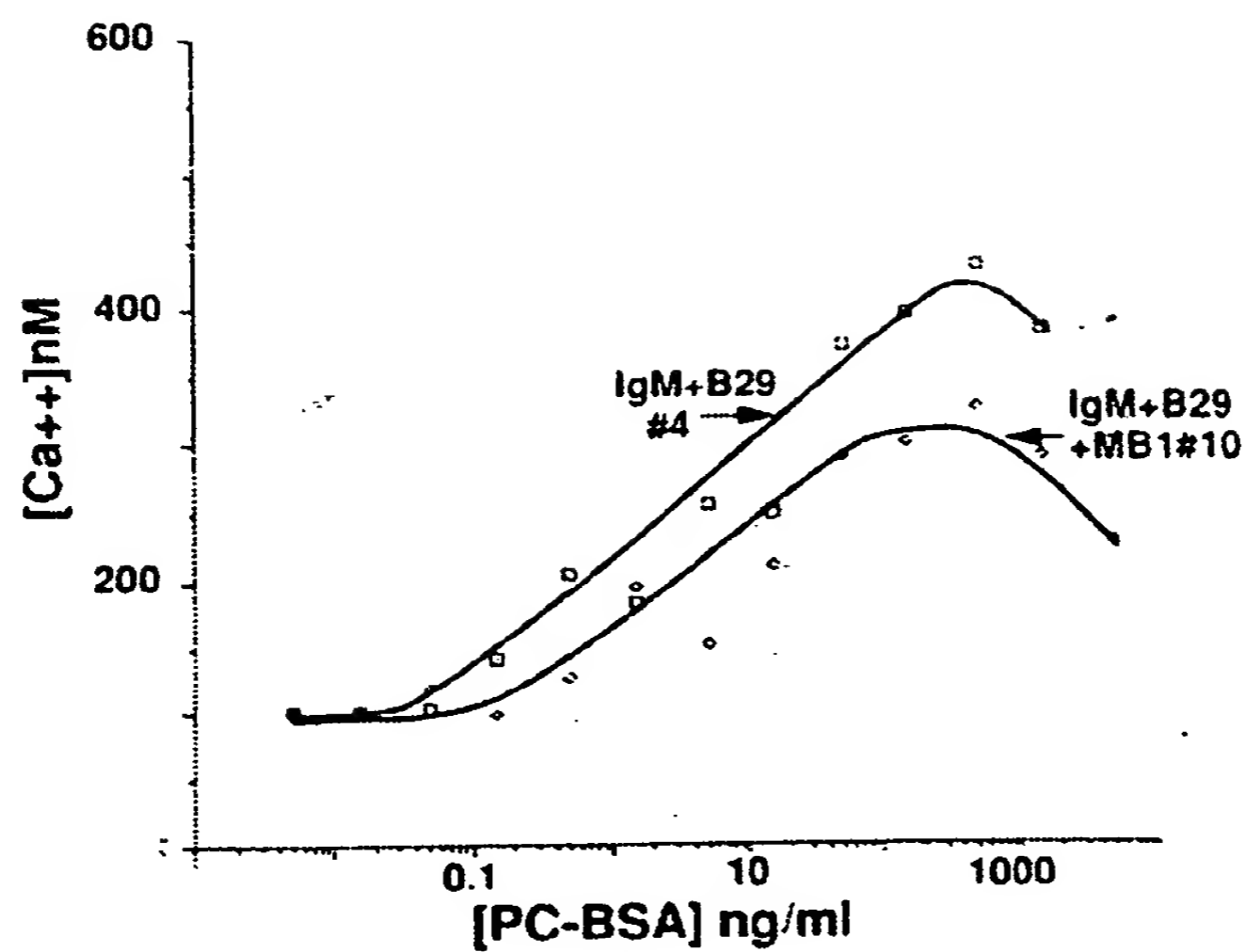


FIG. 8A

PC      PC-BSA      α-CD3  
↓      ↓      ↓

FIG. 8B

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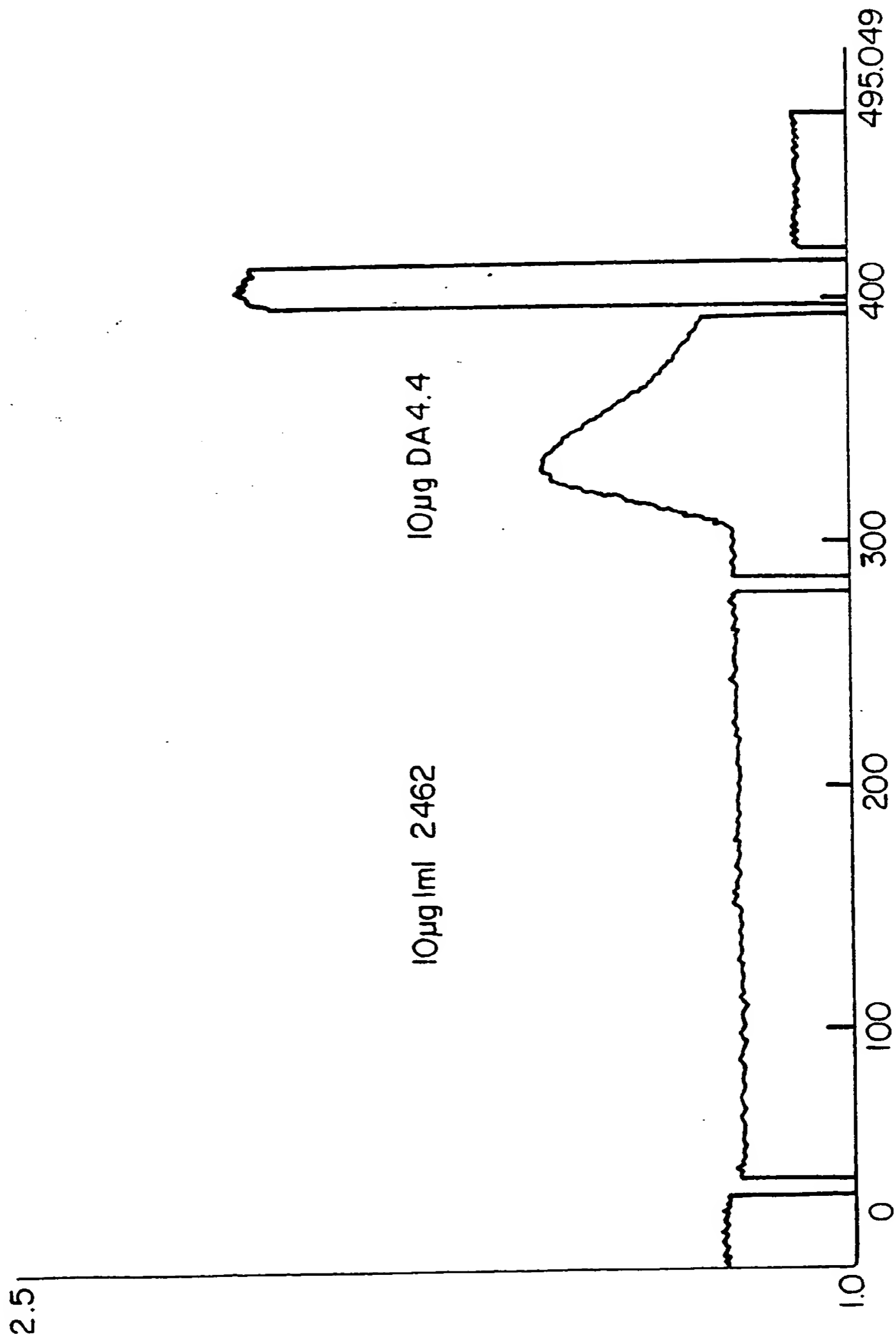


FIG. 9

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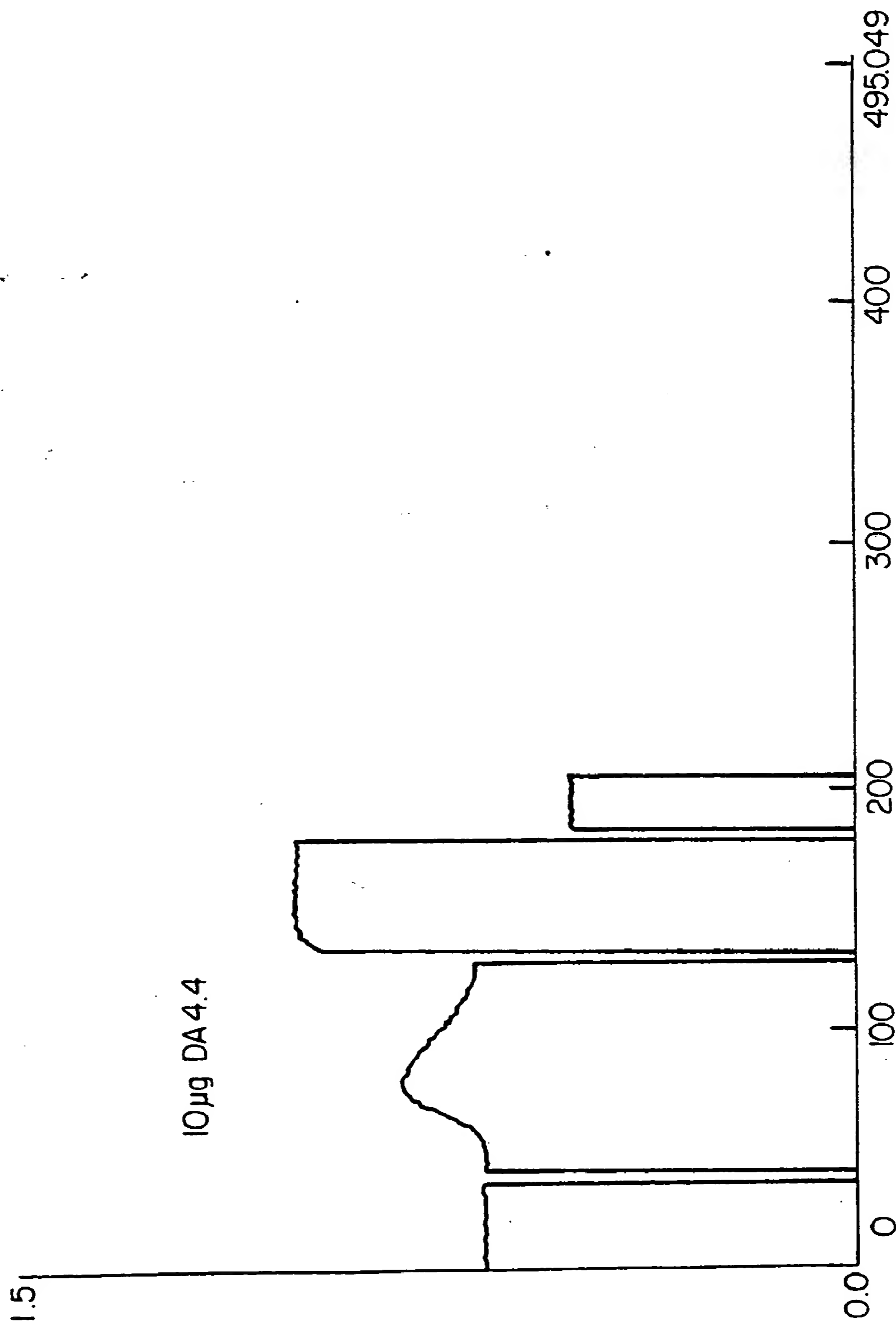


FIG. 10

SUBSTITUTE SHEET

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/01865

|  |  |                                     |
|--|--|-------------------------------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>  |  |                                     |
| According to International Patent Classification (IPC) or to both National Classification and IPC  |  |                                     |
| Int.Cl. 5 C12N15/62;<br>A61K35/14;   | C12P21/08;<br>A61K39/44;   | C12N5/10;<br>G01N33/48<br>A61K37/02 |
| <b>II. FIELDS SEARCHED</b>   |  |                                     |
| Minimum Documentation Searched <sup>7</sup>  |  |                                     |
| Classification System  | Classification Symbols   |                                     |
| Int.Cl. 5  | C12N ; C07K  |                                     |
| Documentation Searched other than Minimum Documentation<br>to the Extent that such Documents are included in the Fields Searched <sup>8</sup>  |  |                                     |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>  |  |                                     |
| Category <sup>10</sup>   | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>   | Relevant to Claim No. <sup>13</sup> |
| X  | NATURE.<br>vol. 343, 22 February 1990, LONDON GB<br>pages 760 - 762<br>Hombach, J. et al.; 'Molecular components<br>of the B-cell antigen receptor complex of<br>the IgM class.'<br>see the whole document<br>---  | 1-6, 23,<br>25, 27,<br>30, 62       |
| X  | PROCEEDINGS OF THE NATIONAL ACADEMY OF<br>SCIENCES OF USA.<br>vol. 89, January 1992, WASHINGTON US<br>pages 633 - 637<br>ISHIHARA, K. ET AL.; 'B29 gene products<br>complex with immunoglobulins on B<br>lymphocytes'<br>see the whole document<br>---<br>-/-- | 12-16                               |
| <p><sup>10</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> |  |                                     |
| <b>IV. CERTIFICATION</b>   |  |                                     |
| Date of the Actual Completion of the International Search  | Date of Mailing of this International Search Report  |                                     |
| 23 JUNE 1993   | 13. 07. 93   |                                     |
| International Searching Authority  | Signature of Authorized Officer  |                                     |
| EUROPEAN PATENT OFFICE   | NAUCHE S.A.  |                                     |

Form PCT/ISA/210 (second sheet) (January 1985)

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |   |                       |
|--|---|-----------------------|
| Category *   | Citation of Document, with indication, where appropriate, of the relevant passages  | Relevant to Claim No. |
| X  | <p>NUCLEIC ACIDS RESEARCH.<br/>vol. 19, no. 15, 1991, ARLINGTON, VIRGINIA<br/>US<br/>pages 4133 - 4137<br/>Hoogenboom, H.R. et al.; 'Multi-subunit on<br/>the surface of filamentous phages :<br/>methodologies for displaying antibody<br/>(Fab) heavy and light chains.'<br/>see the whole document<br/>---</p> | 40,70                 |
| A  | <p>NATURE.<br/>vol. 352, 25 August 1991, LONDON GB<br/>pages 777 - 781<br/>Venkitaraman, A.R. et al.; 'The B-cell<br/>antigen receptor of the five<br/>immunoglobulin classes.'<br/>---</p>   |                       |
| A  | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF<br/>SCIENCES OF USA.<br/>vol. 88, May 1991, WASHINGTON US<br/>pages 3982 - 3986<br/>Campbell, K.S. et al.; 'Igm antigen<br/>receptor complex contains phsphoprotein<br/>products of B29 and mb-1 genes.'<br/>---</p>  |                       |
| P,X  | <p>THE JOURNAL OF EXPERIMENTAL MEDECINE<br/>vol. 175, June 1992, US<br/>pages 1669 - 1676<br/>Costa, T.E. et al.; 'Functional<br/>reconstitution of an Immunoglobulin<br/>antigen receptor in T cells.'<br/>see the whole document<br/>-----</p>  | 1-76                  |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/01865

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 53-58 are directed to a method of treatment of the human/animal body (Rule 39.1(iv) PCT) the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.